Universidade de Aveiro
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Daniela Salomé Moderno do Couto

LIPIDÓMICA DE MICROALGAS PARA IDENTIFICAÇÃO DA SUA ASSINATURA LIPÍDICA E BIOPROSPECÇÃO DE FITOQUÍMICOS BENÉFICOS À SAÚDE

LIPIDOMICS OF MICROALGAE: UNRAVELING THEIR LIPID SIGNATURE AND BIOPROSPECTING BIOACTIVE PHYTOCHEMICALS

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Bioquímica, realizada sob a orientação científica do Professor Doutor Pedro Miguel Dimas Neves Domingues, Professor auxiliar com Agregação do Departamento de Química da Universidade de Aveiro, coorientação científica da Professora Doutora Maria do Rosário Gonçalves dos Reis Marques Domingues, Professora Associada com Agregação do Departamento de Química da Universidade de Aveiro e coorientação científica da Doutora Joana Gabriela Laranjeira da Silva, Diretora da Unidade Orgânica e de R&D da Allmicroalgae Natural Products SA, Portugal.

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Dedico este trabalho aos meus pais,
António Couto & Helena Couto

“Adoramos a perfeição, porque não a podemos ter; repugná-la-íamos se a tivéssemos. O perfeito é o desumano porque o humano é imperfeito.”

Fernando Pessoa, Livro do Desassossego, por Bernardo Soares.

o júri

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	Doutora Narcisa Maria Mestre Bandarra Investigadora Auxiliar do Instituto Português do Mar e da Atmosfera

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“Fazer da gratidão
o nosso chão,
o nosso farol
e a casa do nosso coração.
Confiar que é ela a força
que dá sentido ao caminho.
Agradecer (muito) à vida
pelos dias bonitos
em que a nossa vontade de fazer
Se cruza com a nossa coragem de ser”

Sofia Castro Fernandes, Inspira-te

palavras-chave

Microalgas, lipidómica, lípidos polares, condições de crescimento, heterotrofia, autotrofia, método de extracção de lípidos, fosfolípidos, glicolípidos, quimiotaxonomia

Resumo

As microalgas atraem cada vez mais a atenção dos consumidores, pois são ricas em nutrientes essenciais, tais como lípidos ómega-3 e ómega-6, e o seu consumo tem sido descrito como benéfico para a saúde humana e animal. No entanto, a maioria dos trabalhos publicados sobre a caracterização lipídica de microalgas limita-se a identificar o perfil de ácidos gordos e o conteúdo de outros lípidos abundantes e bioativos como fosfolípidos e glicolípidos, continua a ser pouco conhecido.

De modo a contribuir para aumentar conhecimento nesta área, esta tese teve como objetivo a identificação e comparação do perfil de lípidos polares e de ácidos gordos de microalgas com valor acrescentado, cultivadas industrialmente, utilizando abordagens lipidómicas baseadas em espectrometria de massa e avaliar as atividades biológicas de diversos extratos lipídicos. Assim os objetivos específicos deste trabalho foram: (1) Identificar o lipidoma da microalga *C. vulgaris* crescida em condições de autotrofia e de heterotrofia (designadas respetivamente C-Auto e C-Hetero); (2) Identificar o perfil lipídico de extratos de C-Auto e C-Hetero obtidos com solvente e tecnologia permitida na indústria alimentar (etanol e etanol assistido por sonda de ultra-sons), usados como métodos alternativos aos métodos de extração tradicionais; (3) Identificar a composição lipídica de extratos obtidos de 2 novas estirpes de *C. vulgaris* desenvolvidas por mutagénese química, cultivadas heterotroficamente e aprovadas como suplemento e ingrediente alimentar (designadas como C-White e C-Honey); (4) Identificar o perfil lipídico de 2 espécies de *Nannochloropsis* (*N. oceânica* e *N. limnética*) e avaliar a plasticidade do lipidoma com as condições de crescimento, quando cultivadas no interior e no exterior; (5) Comparar o lipidoma polar de 7 espécies de microalgas pertencentes a 3 diferentes filos, Chlorophyta (*C. vulgaris*, *S. obliquus*, *T. chuii*, *C. amblystomatis*), Ochrophyta (*N. oceanica*, *P. tricornutum*) e Cyanobacteria (*Spirulina* sp.) e avaliar o potencial quimio-taxonómico do lipidoma polar de microalgas; (6) Avaliar *in chemico* as propriedades antioxidantes dos extratos lipídicos das 2 *Nannochloropsis* e das 4 *C. vulgaris* estudadas e as propriedades anti-inflamatórias dos extratos das 4 *C. vulgaris*; (7) Avaliar *in vitro* a atividade anti-inflamatória do extrato lipídico e de frações enriquecidas em fosfolípidos e glicolípidos da *N. oceanica*.

Os resultados obtidos no estudo do lipidoma da *C vulgaris* cultivada em autotrofia e heterotrofia permitiu identificar o perfil de fosfolípidos e glicolípidos destas algas e mostrou que o crescimento autotrófico da *C. vulgaris* induziu um aumento da produção de glicolípidos e de espécies lipídicas esterificadas com ácidos gordos mais insaturados, nomeadamente o ALA. Por outro lado, o crescimento heterotrófico permitiu o aumento do teor em fosfolípidos e de espécies lipídicas com ácidos gordos com menos insaturações, nomeadamente o LA. A composição lipídica dos extratos de C-Auto e C-Hetero extraídos com etanol combinado com sonda de ultrassons foi similar à composição dos extratos extraídos com o método convencional com diclorometano/metanol, pelo que estes extratos de etanol podem ser no futuro utilizados como ingredientes em novos produtos alimentares.

Resumo

A análise do lipidoma da C-White e C-Honey, indica que o lipidoma destas algas incluem as mesmas classes de lípidos que a C-Hetero, com a exceção de 3 classes de lípidos apenas identificadas nestas estirpes (DGGA, PS e CL). Além destas classes, foram também observadas diferenças significativas na abundância relativa da maioria das espécies de lípidos identificadas (~80 %), revelando uma assinatura lipídica específica de cada estirpe.

O estudo da assinatura lipídica da *N. Oceânica* e da *N. Limnética* mostrou que a *N. Oceânica* é mais rica em alguns lípidos betaína e menos rica em liso-lípidos comparativamente com a *N. Limnética*. Verificou se que as condições de crescimento influenciam a composição lipídica, modulando a abundância de algumas das espécies lipídicas. As duas espécies, quando cultivadas no exterior são mais ricas em fosfolípidos e lípidos betaína esterificados com EPA. Por outro lado, o crescimento destas espécies no interior, em condições mais controladas, aumentou o seu teor lipídico, particularmente de glicolípidos.

Após a identificação dos lípidos das algas selecionadas, foi realizada a comparação do seu lipidoma, para avaliar semelhanças e diferenças e se a assinatura lipídica podia ser correlacionada com a classificação taxonómica. A análise lipidómica das 7 espécies de microalgas comercializadas permitiu a identificação de 19 classes lipídicas no total, de classes de fosfolípidos, glicolípidos, betaínas e esfingolípidos, mas apenas 12 foram comuns em todas as algas. A análise lipidómica e análise estatística dos dados obtidos revelaram uma assinatura lipídica específica da espécie de microalga. Também observamos algumas características do lipidoma destas algas que permitiram agrupá-las por filo e por meio aquático (microalgas marinhas e de água doce). Os glicolípidos foram o grupo de lípidos mais conservados nas microalgas pertencentes ao mesmo filo. Por outro lado, os lípidos betaína e os lípidos esterificados com EPA foram os mais abundantes nas microalgas marinhas, enquanto o lipidoma das microalgas de água doce apresentam uma maior quantidade de lípidos esterificados com ALA.

Relativamente à bioprospecção dos extratos lipídicos verificou-se que todos os extratos tinham potencial antioxidante, determinado através de eliminação de radicais DPPH$^{\bullet}$ e ABTS$^{\bullet+}$, com a C-Auto e C-Hetero a deterem o maior potencial. Quanto ao potencial de inibição da Cox-2, estras microalgas da espécie *C. vulgaris* também apresentaram uma maior atividade anti-inflamatória. A atividade antioxidante dos extratos de *C. vulgaris* extraídos com etanol e ultrassons, foi semelhante aos extratos obtidos com os métodos convencionais usando solventes clorados. Quer o extrato da *N. oceanica* quer as suas frações demonstraram potencial anti-inflamatório, determinado pela diminuição dos níveis de NO e da expressão de genes pró-inflamatórios em macrófagos estimulados por LPS.

Assim, este trabalho permitiu identificar pela primeira vez o lipidoma de microalgas com aplicação industrial, mostrando que o lipidoma é específico de cada espécie, embora se possam ser observadas algumas características típicas do filo ou de espécies de água doce ou salgada. Foi ainda demonstrado que o lipidoma pode ser modelado pelas condições de crescimento, e que os extratos lipídicos têm propriedades bioativas, nomeadamente antioxidante e anti infamatória. Estes resultados podem contribuir para o desenvolvimento de novos produtos baseados em microalgas para aplicação nas indústrias alimentares, de nutracêutica ou de suplementos, ente outras.

keywords

Microalgae, lipidomics, polar lipids, growth conditions, heterotrophy, autotrophy, lipid extraction method, phospholipids, glycolipids, chemotaxonomy

abstract

Microalgae have increasingly attracted the attention of consumers because they are rich in essential nutrients, such as omega-3 and omega-6 lipids, and their consumption has been described as beneficial to human and animal health. However, the majority of the published works on the lipid characterization of microalgae, described the identification of fatty acids profile, and other abundant and bioactive lipids such as phospholipids and glycolipids are little known. Thus, this thesis had the objectives of identifying and comparing the polar lipid and fatty acid profiles of industrially cultivated microalgae with added value, using modern lipidomic approaches based on mass spectrometry and evaluating their biological activities. The specific goals were to (1) Identify the lipidome of the microalgae *C. vulgaris* grown under autotrophic and heterotrophic conditions (designated respectively C-Auto and C-Hetero); (2) Identify the lipid profile of C-Auto and C-Hetero extracts extracted with a solvent and technology allowed in the food industry (ethanol and ethanol assisted by ultrasound probe), used as alternative methods to traditional extraction methods; (3) Identify the lipid composition of extracts of 2 new strains of *C. vulgaris* developed by chemically-induced random mutagenesis, and cultured heterotrophically. These new strains are approved as a food supplement and food ingredient (herein designated as C-White and C-Honey); (4) Identify the lipid profile of 2 species of *Nannochloropsis* (*N. oceanica* and *N. limnetica*) and evaluate their plasticity with growing conditions (indoors and outdoors); (5) Compare the polar lipidome of 7 species of microalgae belonging to 3 different phyla, Chlorophyta (*C. vulgaris*, *S. obliquus*, *T. chuii*, *C. amblystomatis*), Ochrophyta (*N. oceanica*, *P. tricornutum*) and Cyanobacteria (*Spirulina* sp) and evaluate the chemotaxonomic potential of the microalgae polar lipids; (6) Evaluate *in chemico* the antioxidant properties of lipid extracts from the 2 *Nannochloropsis* and the 4 *C. vulgaris* strains and the anti-inflammatory properties of the 4 *C. vulgaris* strains. (7) Evaluate *in vitro* the anti-inflammatory activity of lipid extract and fractions enriched in phospholipids and glycolipids from *N. oceanica*.

The results obtained in the study of the lipidome of *C. vulgaris* grown in autotrophy and heterotrophy allowed to identify the profile of phospholipids and glycolipids of these algae and showed that the autotrophic growth of *C. vulgaris* induced an increase in the production of glycolipids and lipid species esterified with more unsaturated fatty acids, namely ALA. On the other hand, the heterotrophic growth increased the content of phospholipids and lipid species with less unsaturated fatty acids, namely LA. The lipid composition of the C-Auto and C-Hetero extracts extracted with ethanol and ultrasounds probe was similar to the lipid composition of the extracts extracted with the conventional dichloromethane/methanol method, so these UAE ethanol extracts can be used in the future as ingredients in new food products.

abstract

The lipidome analysis of the C-White and C-Honey indicates that the lipidome of these algae includes the same lipid classes as C-Hetero, except for 3 lipid classes only identified in these new strains (DGGA, PS and CL). In addition to these classes, it was also observed significant differences in the relative abundance of most lipid species identified (~80 %) in both strains, revealing a strain-specific lipid signature.

The lipid signature of *N. oceanica* and *N. limnetica* were also evaluated using modern lipidomics approaches. The results obtained showed that *N. Oceanica* is richer in some betaine lipids and less rich in lysolipids compared to *N. limnetica*. It also was found that growth conditions influence the lipid composition, by modulating the abundance of some lipid species. Both species grown outdoors were richer in phospholipids and betaine lipids esterified with EPA, but when grown indoors their lipid content increased, particularly of glycolipids.

After lipid identification of the selected algae, a comparison of their lipidome was performed to assess similarities and differences and whether this lipid signature had any correlation with taxonomic classification. Lipidomic analysis of the 7 commercialized microalgae species allowed the identification of a total of 19 lipid classes, from phospholipid, glycolipid, betaine, and sphingolipid, but only 12 classes were common in all algae. Lipidomic analysis and statistical analysis of the data revealed a species-specific lipid signature of the microalgae. However, some characteristics of the lipidome were observed that allowed the classifying of the algae by phylum and marine or freshwater environments. Glycolipids were the most conserved group of lipids of microalgae belonging to the same phylum. On the other hand, betaine lipids and EPA-esterified lipids were the most abundant lipids in marine microalgae, while the lipidome of freshwater microalgae was characterized by a higher amount of lipids esterified with ALA.

Regarding the bioprospecting of the lipid extracts, it was found that all the extracts had antioxidant potential measured by DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging activities, with C-Auto and C-Hetero having the highest antioxidant potential. As for the Cox-2 inhibition potential, C-Auto and C-Hetero also showed higher anti-inflammatory activity. It was also observed that the antioxidant activity of C-Auto and C-Hetero extracts extracted with ethanol and ultrasound, was similar to extracts obtained with conventional methods using chlorinated solvents. Both N. oceanica total lipid extract and fractions showed anti-inflammatory potential by decreasing NO levels and expression of proinflammatory genes in LPS-stimulated macrophages.

Thus, this work allowed the identification, for the first time, of the lipidome of microalgae with industrial application, showing that the lipidome is species-specific, although we could observe some characteristics which were typical of the phylum or the environment (freshwater or saltwater species). It was also shown that the lipidome can be modulated by growth conditions, and that lipid extracts have bioactive properties, namely antioxidant and anti-inflammatory properties. These results may contribute to the development of new microalgae-based products for application in the food, nutraceutical or supplement industries, among others.

Publications and communications

The work developed during this thesis resulted in 3 publications and 2 manuscripts submitted (as the first author) as well as another 10 publications (as co-author) in international scientific journals with Referee. And also 2 oral and 4 poster communications (as the first author) in national and international meetings.

Publications in international scientific journals with Referee

Couto, D., Melo, T., Conde, T. A., Costa, M., Silva, J., Domingues, M. R. M., Domingues, P. (2021). Chemoplasticity of the polar lipid profile of the microalgae *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions. Algal Research, 53, 102128. https://doi.org/10.1016/j.algal.2020.102128.

Couto, D., Melo, T., Conde, T. A., Moreira, A. S., Ferreira, P., Costa, M., Silva, J., Domingues, M. R. M., Domingues, P. (2022). Food grade extraction of *Chlorella vulgaris* polar lipids: A comparative lipidomic study. *Food Chemistry*, 375, 131685. https://doi.org/10.1016/j.foodchem.2021.131685.

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*Both authors contributed equally to the manuscript.

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*Both authors contributed equally to the manuscript.

Oral communications

Couto, D., Melo, T., Conde, T. A., Costa, M., Silva, J., Domingues, M. R. M., Domingues, P. Unraveling the effects of growth conditions on the polar lipid profile of *Chlorella Vulgaris*. Lipids in the Ocean 2nd edition. University of Aveiro (Portugal), 2020/11/18.

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Poster communications

Couto, D., Melo, T., Silva, J., Domingues, P., Domingues, M. R. M. A comparative analysis of the lipid content of *Chlorella* sp. Grown under autotrophic or heterotrophic conditions using a LC-MS lipidomic approach. Conferência APAA (Associação Portuguesa de Algologia Aplicada 2019), 2019. Matosinhos.

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Couto, D., Conde, T. A., Melo, T., Neves, B., Costa, M., Silva, J., Domingues, M. R. M., Domingues, P. Fluctuations of polar lipids species-rich in EPA from two strains of *Nannochloropsis* sp. grown in the laboratory and outdoor flat-panels. Gerli 16th Lipidomics Meeting, 26-29 September 2021, Bordeux, France.

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List of abbreviations

AA	Arachidonic acid
ABTS	2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AGC	Automatic gain control
AI	Atherogenic index
ALA	Alpha-linolenic acid
Anth	antheraxanthin
AST	astaxanthin
BL	Betaine lipid
BLL	Betaine-like lipids
C.a	*Chlorococcum amblystomatis*
C.v	*Chlorella vulgaris*
C:N	Number of acyl carbons: number of double bonds
C-Auto	*Chlorella vulgaris* grown under autotrophic conditions
CE	Collision energy
Cer	Ceramide
Cer-1P	Ceramide-1-phosphate
CH_2Cl_2	Dichloromethane
CH_3CN	Acetonitrile
CH_3COO^-	Acetate anion
CH_3COOCH_3	Methyl acetate
$CHCl_3$	Chloroform
C-Hetero	*Chlorella vulgaris* grown under heterotrophic conditions
Chla	Chlorophyll a
Chlb	Chlorophyll b
C-Honey	*Chlorella vulgaris* strain with yellow colour
CID	Collision-induced dissociation
CL	Cardiolipin
CO_2	Carbon dioxide
CoA	Coenzyme A
COX-2	Cyclooxygenase-2
C-White	*Chlorella vulgaris* strain with white colour
DA	Alzheimer disease
DAG	Diacylglycerol
DCM	Dichloromethane:methanol (2:1, v/v)
DGAT	Diacylglycerol acyltransferase
DGCC	Diacylglyceryl carboxyhydroxymethylcholine
DGDG	Digalactosyldiacylglycerol
DGGA	Diacylglycerylglucuronide
DGMG	Digalactosylmonoacylglycerol
DGTA	1,2-diacylglyceryl-3-O-2'-(hydroxymethyl)-(N,N,N-trimethyl)-β-alanine

DGTS	Diacylglyceryl trimethyl homoserine
DHA	Docosahexaenoic acid
Dim	Dimensions
DMSO	Dimethyl sulfoxide
DPPH	α,α-diphenyl-β-picrylhydrazyl
DW	Dry weight
EA	Early antigen
EBV	Epstein-Barr virus
EFSA	European Food Safety Authority
EMS	Ethyl methanesulfonate
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
ESBL	Extended-spectrum β-lactamase
ESI	Electrospay ionization
EtOH	Ethanol
EY	Extract yield
FA	Fatty acid
FAME	Fatty acid methyl ester
FDA	Food and Drug Administration
FFA	Free fatty acid
FID	Flame-ionization detection
FP	Flat Panel
FTIR	Fourier-transform infrared spectroscopy
G3P	Glycerol-3-phosphate
GalCer	Galactosylceramide
GC	Gas chromatography
GL	Glycolipid
GL	Glycolipid
GlcADG	Glucuronosyldiacylglycerol
GlcCer	Glucosylceramide
GPAT	Glycerol-3-phosphate acyltransferase
GRAS	Generally Recognized As Safe
h/H	Hypocholesterolemic/ hypercholesterolemic index
H_2O	Water
H_3PO_4	Phosphoric acid
HCA	Hierarchical cluster analysis
$HCOO^-$	Formate anion
HDL	High-density lipoprotein
HexCer	Hexosylceramide
hGSL	Host glycosphingolipid
HILIC	Hydrophilic interaction liquid chromatography
HPLC	High-performance liquid chromatography
IC	Inhibitory concentration
IL	Interleukin

IMTA	Integrated multi-trophic aquaculture
iNOS	Inducible nitric oxide synthase
IT	Injection time
KCl	Potassium chloride
LA	Linoleic acid
LC	Liquid chromatography
LCE	Lipid content in the extract
LLE	Liquid-liquid extraction
LPA	Lysophosphatidic acid
LPAAT	Lysophosphatidic acid acyltransferase
LPC	Lysophosphatidylcholine
LPE	Lysophosphatidylethanolamine
LPG	Lysophosphatidylglycerol
LPI	Lysophosphatidylinositol
LPS	Lipopolysaccharide
LY	Lipid yield
m/z	Mass-to-charge ratio
MAG	Monoacylglycerol
MALDI	Matrix-assisted laser desorption/ionization
MeOH	Methanol
MEP	Methyl erythritol phosphate
MGCC	Monoacylglycerylcarboxyhydroxymethylcholine
MGDG	Monogalactosyldiacylglycerol
MGMG	Monogalactosylmonoacylglycerol
MGTA	Monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine
MGTS	Monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine
MMPE	Monomethylphosphatidylethanolamine
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MTBE	Methyl-tert-butyl ether
MUFA	Monounsaturated fatty acid
N.o	*Nannochloropsis oceanica*
n-3	Omega-3
n-6	Omega-6
nC	Total number of carbon atoms on the esterified fatty acids
nDD	Total number of double bonds
Neo	neoxanthin
NF-κb	Factor nuclear kappa B
NL-In	*N. limnetica* grown indoors
NL-Out	*N. limnetica* grown outdoors
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NO-In	*N. oceanica* grown indoors
NO-Out	*N. oceanica* grown outdoors

NP	Normal phase
NVI	Nutritive value
OxPL	Oxidized phospholipid
P.t	*Phaeodactylum tricornutum*
PA	Phosphatidic acid
PAP	Phosphatidic acid phosphatase
PBR	Photobioreactor
PC	Phosphatidylcholine
PCA	Principal Component Analysis
PDAT	Phospholipid:diacylglycerol acyltransferase
PDPT	Phosphatidyldimethylpropanethiol
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
Pheoa	Pheophorbide a
Pheob	Pheophorbide b
Phya	Pheophytin a
Phyb	Pheophytin b
PI	Phosphatidylinositol
PI-Cer	Inositolphosphoceramide
PL	Phospholipid
PLB	Prolamellar body
PLS-DA	Partial Least Squares-Discriminant Analysis
PON1	Paraoxonase 1
PPAR-y	Peroxisome proliferator-activated receptor gamma
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acid
QC	Glutaminyl cyclase
Q-TOF	Quadrupole-Time of Flight
RA	Relative abundance
RAW 264.7	Mouse leukaemic monocyte macrophage cell line
$RCOO^-$	Carboxylate anion
rePON1	Recombinant Paraoxonase 1
RIC	Reconstructed ion chromatogram
RP	Reverse phase
S.o	*Scenedesmus obliquus*
SD	Standard deviation
SDG	Sustainable development goals
SEM	Scanning electron microscopy
SFA	Saturated fatty acid
SFC	Supercritical fluid chromatography
sGSL	Sialic acid glycosphingolipid
SL	Sphingolipid
SLE	Solid-liquid extraction
SP	*Spirulina* sp.

sp.	Species not specified
SPE	Solid-phase extraction
spp.	Several species
SQDG	Sulfoquinovosyldiacylglycerol
SQMG	Sulfoquinovosylmonoacylglycerol
T.c	*Tetraselmis chuii*
TAG	Triacylglycerol
TE	Trolox equivalents
THP1	Human leukaemia monocytic cell line
TI	Thrombogenic index
TLC	Thin-layer chromatography
TNFα	Tumour necrosis factor α
TPA	12-O-Tetradecanoylphorbol-13-acetate
Trolox	6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
UAE	Ethanol extraction assisted by an ultrasound probe
UDP	Uridine-5'-diphosphate
UPLC	Ultra-performance liquid chromatography
UV-Vis	Ultraviolet-visible
Viola	violaxanthin
13S-HODE	(9Z,11E,13S)-13-hydroxyoctadeca-9,11-dienoic acid
13S-HOTE	(9Z,11E,13S,15Z)-13-hydroxyoctadeca-9,11,15-trienoic acid
15S-HEPE	(5Z,8Z,11Z,13E,15S,17Z)-15-hydroxyeicosa-5,8,11,13,17-pentaenoic acid

CHAPTER 1 - Introduction

Parts of this introduction (in subchapter 4) were written collaboratively and were published in a review paper of which I am a co-author: “Rey, F., Melo, T., Lopes, D., Couto, D., Marques, F., Domingues, M. R. M., (2022). Applications of lipidomics in marine organisms: Progresses, challenges and future perspectives. Molecular Omics, 18, 357-386. https://doi.org/10.1039/D2MO00012A”

1. Microalgae: a sustainable source of high-value phytochemicals

Microalgae are important biological and sustainable resources and are gaining interest for a wide range of applications, such as food, feed, nutraceuticals, and other biotechnological applications. They are considered a promising source of nutrients and are recommended for sustainable and healthy diets.

The *Nostoc* sp. is a dark blue-green Cyanobacteria and is pointed as the first microalgae used by humans around 2000 years ago for food, feed, remedies to fight disease or as organic fertilizers in agriculture [1]. Later *Chlorella* sp. and *Spirulina* sp. were used as foods in Taiwan, Japan and Mexico [1]. Nowadays several microalgae are being produced and also evaluated for novel food or a source of health and bioactive ingredients for food supplements [2]. They are consumed worldwide and have attracted attention as a source of nutrients and bioactive phytochemicals such as polyunsaturated fatty acids (PUFAs) that are rarely found in terrestrial plants [3]. They are highlighted as sustainable sources of *n*-3 PUFAs (EPA, DHA), an alternative to fish, a limited resource that nowadays remains the main provider of these essential FAs for diet [3] Algae-based products allow addressing the increasing need for society and industries to replace chemical and animal ingredients by ingredient of vegetable origin. Microalgae are produced in sustainable aquaculture systems, and their ability to capture CO_2, fix nitrogen and convert sunlight, water and nutrients into valuable compounds are boosting the interest in the exploitation of these micro factories for industries, keeping planet boundaries and mitigating climate changes.

Microalgae are unicellular algae and predominantly photosynthetic organisms [4], found in marine, freshwater and terrestrial habitats [1]. It is an extremely varied group, distributed across diverse ecosystems. Thousands of species of microalgae have been identified [1] with different metabolisms (autotrophic, heterotrophic and mixotrophic) [5]. They can be classified based on their pigmentation into four main groups: green (phylum Chlorophyta), red (phylum Rhodophyta), brown (phylum Ochrophyta) and blue-green (phylum Cyanobacteria) [6]. Microalgae also can be divided into prokaryotic and eukaryotic organisms [7]. Prokaryote microalgae comprise a single phylum, Cyanobacteria, while Eukaryota includes ten phyla: Chlorophyta, Rhodophyta, Ochrophyta, Euglenozoa, Cryptista, Haptophyta, Myzozoa, Cercozoa, Glaucophyta, and Charophyta (**Figure 1.1**).

This phylogenetic and evolutionary variety implies a high biotechnological potential, as a source of biomass, nutrients, and ingredients for a plethora of applications.

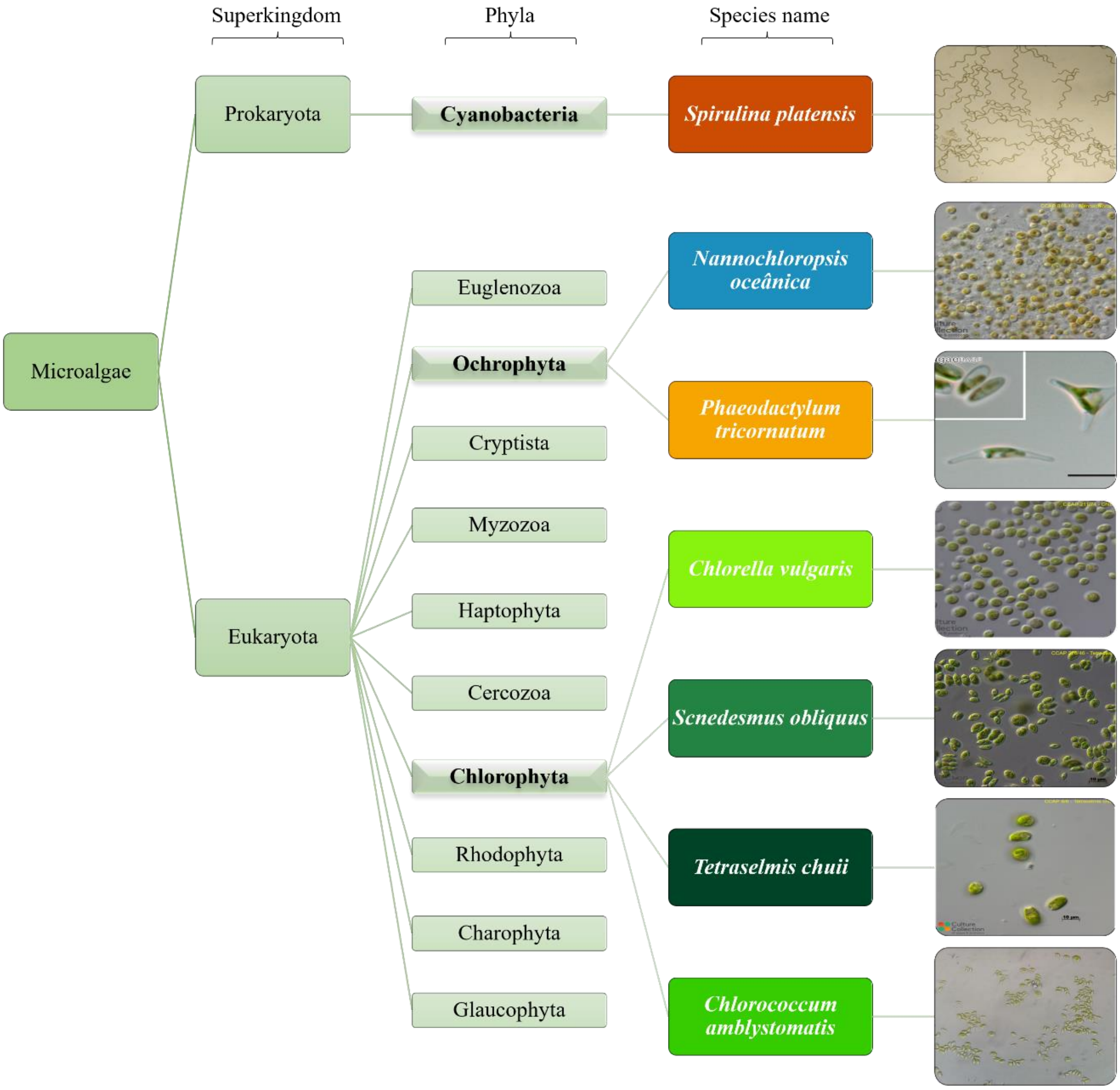

Figure 1.1. Classification of microalgae with illustrations of species used in this thesis.

The microalgae are mainly composed of lipids, proteins, carbohydrates and nucleic acids and lower amounts of vitamins (B1, B2, B3, B6, B12, E, K, D, among others) [8,9]. The reported total content of proteins, carbohydrates and lipids of the microalgae used in this thesis: (four species of Chlorophyta (*Chlorella vulgaris*, *Scenedesmus obliquus*, *Tetraselmis chuii*, *Chlorococcum amblystomatis*) three of Ochrophyta (*Nannochloropsis limnetica*, *Nannochloropsis oceanica*, *Phaeodactylum tricornutum*) and one of Cyanobacteria

(*Spirulina* sp.) phyla are shown in **Table 1.1**. Among them, *Nannochloropsis oceanica* showed the higher lipid content (18-46 % of DW), followed by *Chlorococcum amblystomatis* (17-31 % of DW) and *Nannochloropsis limnetica* (24 % of DW).

Table 1.1. Biochemical composition of the microalgae species used in this thesis.

	Content range (% of DW)			
Species name	**Proteins**	**Carbohydrates**	**Lipids**	**Refs.**
Chlorella vulgaris	51–58	12–17	8–22	[10–12]
Scenedesmus obliquus	48–56	10–17	11–14	[10,11,13]
Tetraselmis chuii	31–46	25	6–12	[11,13]
Chlorococcum amblystomatis	48–56	6–18	17–31	[13,14]
Nannochloropsis oceanica	10–28	8–27	18-46	[15]
Nannochloropsis limnetica	~37	10	24	[16]
Phaeodactylum tricornutum	35	17	13-16	[11,13]
Spirulina platensis	46–63	8–14	4–9	[10]

In addition to these microalgae species, there are oleaginous microalgae that are very used for their high lipid content that can range from 20 % to 90 % of their dry weight (DW) [17,18] depending on algae species as well as growth and/or environmental conditions. Thus, cultivated conditions are sometimes manipulated to enhance the lipid content and or produce specific lipids. In fact it has been shown that lipid composition depend on the growth phase [19–21], the culture medium [22], stress conditions such as: light [23,24], temperature [24–28], changing nutrient concentration or deplete [23,29,30], (nitrogen [25,29–31], phosphate [29,31], nitrate [26], iron [22]) and salinity [25,32,33]. Nowadays, microalgae are already used either as food or as ingredients in food products, due to their rich composition in lipids, namely the *n*-3 and *n*-6 essential PUFAs which have great nutritional value and benefits for health [34,35]. In addition to PUFAs, some authors have also reported the bioactivity of other groups of lipids present in lipid extracts from microalgae, such as polar lipids, fatty acid methyl esters (FAME) and oxylipins, namely with effects on enzyme activities [36–38] and antioxidant [13,39,40], antimicrobial [41–43], anti-tumour [44–48] and anti-inflammatory activities [49–55]. The bioactivity of these lipids seems to be correlated with their structure, but this is scarcely studied, especially because the detailed lipid composition of microalgae is little identified. The structural characterization of the lipid profile of microalgae has only been achieved by the lipidomics approach, based on liquid chromatography-mass spectrometry (LC-MS) for a few ones, as reviewed in [56,57].

In this Ph.D., lipidomic approaches based on LC-MS were used to provide insights into the polar lipid composition of microalgae and the lipid changes that occur in microalgae grown in different cultivation conditions. Previous studies have shown that the lipidome of microalgae comprises hundreds of lipid species belonging to different lipid classes. These groups of lipids are synthesized by several metabolic pathways and have different biological functions and subcellular locations. In subchapter 1.1 of this introduction, we will provide an overview of the biosynthesis, localization, and function of the lipid classes identified in microalgae. In this work, the characterization and quantification of the lipids of microalgae have been accomplished by lipidomic approaches based on LC-MS. Subchapter 1.2 describes all the steps that constitute the typical workflow of a lipidomic analysis and subchapter 1.3 provides the state-of-the-art of existing lipidomics studies in microalgae, from 2015 to 2021. In this Ph.D., we used *in chemico* assays to evaluate the antioxidant and anti-inflammatory activities of the lipid extracts of microalgae. Subchapter 1.4 describes the studies that reported the potential antioxidant and anti-inflammatory of lipids from microalgae.

1.1. Structural diversity, subcellular localization, and function of lipids in microalgae

The lipidome of microalgae comprises several hundreds of lipids. Lipids are found in cells and organelles membranes, where they play crucial functions as structural components of membranes, as signalling molecules and as a source of energy [58]. Their biological functions and localization on cells are intimately correlated with their chemical structure. Based on their chemical structure, they can be categorized into neutral and polar lipids, containing distinct groups and can be classified into different classes of lipids. This chapter describes the structure, subcellular localization, functions, and pathways of the biosynthesis of lipid groups identified in microalgae: neutral lipids (that can be classified into fatty acids, sterols, and neutral glycerolipids) and polar lipids (that can be classified into phospholipids, betaine lipids, glycolipids, sphingolipids).

1.1.1. Neutral lipids identified in microalgae

1.1.1.1. Fatty acids

Fatty acids (FAs) are the simplest lipids composed of a carboxyl group and an aliphatic carbon chain and are synthesized de novo in the stroma of the chloroplasts [59]. These lipids can have different chain lengths (*e.g.* C6 to C>20) and different unsaturation (number of double bonds): from zero (saturated FAs (SFAs)), one (monounsaturated FAs (MUFAs)), or more than one (polyunsaturated FAs (PUFAs)) [60–62]. The position of the double bond from the methyl terminal in the carbon chain determines the FA nomenclature: in *n*-3 (positioned at C3-C4), *n*-5 (C5-C6), *n*-6 (C6-C7), *n*-7 (C7-C8), *n*-9 (C9-C10), etc [61,63]. In microalgae, a variety of 135 different FAs has been identified [61]. Among them, the most common are the FAs with long carbon chains namely C16 and C18 [60,64]. FAs with odd-chain, hydroxylated, branched-chain, and methylated FAs it has also been found, however typically with low abundance [61]. FA profile of microalgae may vary with species [13] and growth conditions [12], however, it has been reported that the FA profile of microalgae can reflect the phylogenetic relationship at the phylum level [65]. The most predominant FAs are: C16:0, C16:4, C18:3 *n*-3 in Chlorophyta; C16:1 *n*-7, C16:0, C20:5 *n*-3, C14:0 in Ochrophyta; C16:0, C18:4, C18:5, C20:4 *n*-6, C22:6 *n*-3 in Myzozoa); C14:0, C16:0, C18:1 *n*-9 in Haptophyta; C16:0, C18:0, C18:2 *n*-6, C18:4, C20:5 *n*-3 and C22:6 *n*-3 in Cryptophyta; C16:0, C18:2 *n*-6, C20:4 *n*-6 and C20:5 *n*-3 (in Rhodophyta) and C16:1 *n*-7, C18:2 *n*-6 and C18:3 *n*-3 in Cyanobacteria [64]. Most microalgae are rich in essential FAs, that cannot be synthesized by humans, like alpha-linolenic acid (ALA, C18:3 *n*-3) [66] and linoleic acid (LA, C18:2 *n*-6), but also other beneficial *n*-3 and *n*-6 PUFAs, such as arachidonic acid (AA, 20:4 *n*-6), eicosapentaenoic acid (EPA, C20:5 *n*-3) and docosahexaenoic acid (DHA, C22:6 *n*-3) [64,66,67]. A schematic representation of the biosynthesis pathways of long-chain PUFAs by microalgae can be found in **Figure 1.2**. FAs can be found in free form (free fatty acids FFAs) but most of them are attached to neutral or polar lipids.

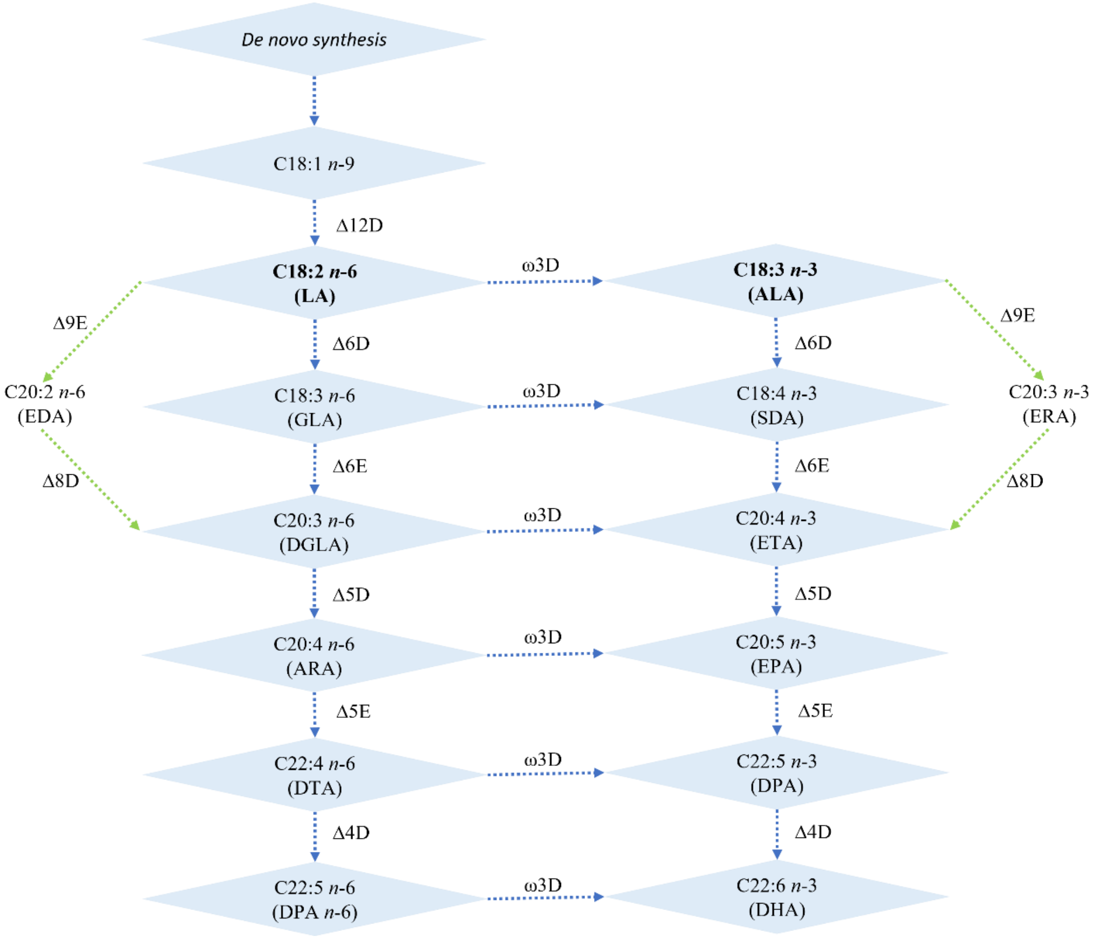

Figure 1.2. Pathways of the biosynthesis of long-chain polyunsaturated fatty acids in microalgae. Abbreviations: D, desaturase; E, elongase, (adapted from [68]).

1.1.1.2. Sterol lipids

Sterol lipids consist of a cyclopentaphenanthrene ring and an alkyl side chain at the C-17 carbon atom [69,70]. They regulate the fluidity and permeability of the membrane and acts as signalling molecules [69]. In microalgae, its synthesis generally can occur by two distinct pathways: the mevalonic acid pathway, in the cytosol; and the methyl erythritol phosphate (MEP) pathway, in the plastid [68]. The content of sterol in microalgae varies according to species and growth conditions [69] (*e.g.* salinity [71] and temperature [72]). Few studies have compared the sterols content of microalgae [73]. In a study that pinpointed the sterol concentration of seventeen different species, it was reported that the *Monoraphidium minutum* was the highest sterol producer (15.3 ± 1.1 µg mg C^{-1}) and *Gomphonema parvulum* was the lowest (2.1 ± 0.2 µg mg C^{-1}) [70]. In another two studies [66,73], *Pavlova lutheri* were identified by far, as the top phytosterol producers (2.6 mg/g dry weight [73] and 97 ± 3 mg/g oil [66]) in a total of ten and nine microalgae, respectively. Regarding the structural diversity of microalgae-derived sterols, they are mainly classified into four groups: 4-desmethyl-Δ^5-sterols; 4-desmethyl-Δ^7-sterols; 4-methylsterols; and di-hydroxylated sterols [74]. Among them, the most predominant are the 4-desmethyl-Δ^5-sterols, such as brassicasterol, campesterol, β-sitosterol and stigmasterol [69,74–76]. The sterol profile of microalgae has been characterized for several species belonging to almost all phyla (namely Cyanobacteria [74], Euglenozoa, Haptophyta, Myzozoa, Ochrophyta, Cercozoa, Glaucophyta, Rhodophyta, and Chlorophyta phyla [77]). Species belonging to the same phyla have been characterized by the presence of characteristic sterols such as 24-ethylcholesterol and epibrassicasterol found in Cyanobacteria [75] and Cryptophytes [78], respectively, although the composition of the sterol may vary with strain [74]. In addition to free sterols, it has been reported the presence of conjugated forms of sterols in microalgae, namely steryl esters (sterol esterified to FA), acyl steryl glycosides (sugar moiety is acylated with a FA), and steryl glycosides (bound to sugar with a glycosidic bond), but with commonly lower concentration that of free sterols [76,77].

1.1.1.3. Neutral glycerolipids

The neutral glycerolipids are constituted by a glycerol molecule linked by an ester linkage to one [monoacylglycerols (MAG)], two [diacylglycerols (DAG)] or three [triacylglycerols (TAG)] fatty acids. In most microalgae, the amount of neutral glycerolipids represents 5%–51% of total lipids [79], in which TAGs are the most abundant ones [62].

TAGs are usually the most abundant in algae and are energy-storage molecules. Its synthesis mostly occurs in the endoplasmic reticulum (ER) via the “eukaryotic” pathway [80,81], however, in microalgae *Chlamydomonas reinhardtii* the “prokaryotic” pathway, that occurs in the chloroplast, was also proposed [82]. The major TAG biosynthesis pathways are the acyl-CoA-dependent pathway and the acyl-CoA-independent pathway [83]. The acyl-CoA-dependent pathway begins with the biosynthesis of LPA through the acylation of glycerol-3-phosphate (G3P) by acyl-CoA:glycerol-3-phosphate acyltransferase (GPAT). LPA is esterified to form PA, by acyl-CoA-dependent acyl-CoA:LPA acyltransferase (LPAAT), which is subsequently dephosphorylated by phosphatidic acid phosphatase (PAP) to form DAG (diacylglycerol). Finally, DAG is esterified to form TAG by the acyl-CoA:DAG acyltransferase (DGAT). In the acyl-CoA-independent pathway, DAG is esterified to form TAG by phospholipid:DAG acyltransferases (PDATs). It has been suggested that both pathways (“eukaryotic” or “prokaryotic”) determine the FA chain in the *sn*-2 position of a glycerol molecule, *i.e.* the glycerolipids derived from “eukaryotic” pathway have FA with C18 chain, while the glycerolipids derived from “prokaryotic” pathway have FA with C16 chain (**Figure 1.3**) [81].

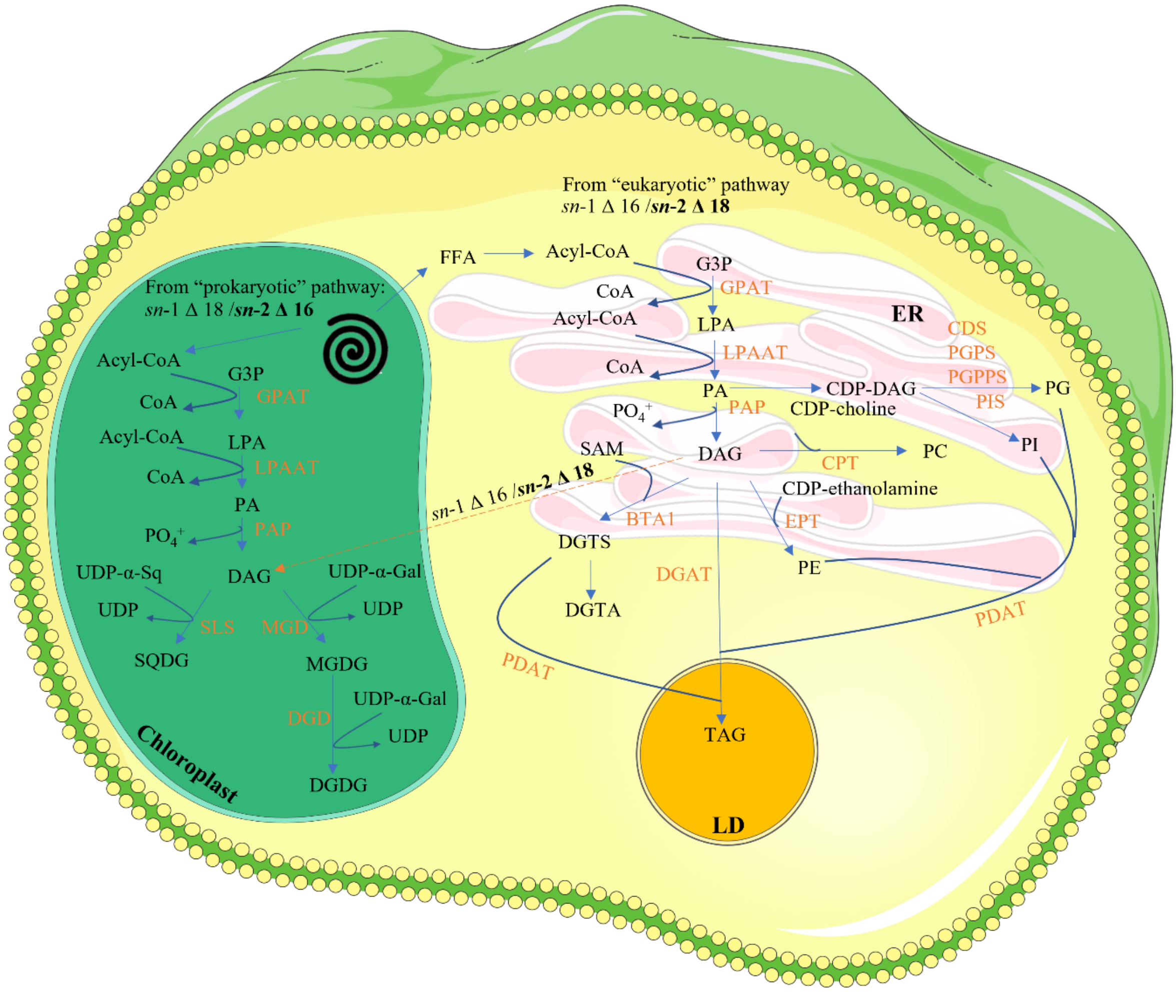

Figure 1.3. Pathways of the biosynthesis of complex lipids in microalgae. Abbreviations: G3P, glycerol-3-phosphate; GPAT, glycerol 3-phosphate acyltransferase; LPAAT, lysophosphatidic acid acyltransferase; PA, phosphatidic acid; DAG, diacylglycerol; PDAT, phospholipid:diacylglycerol acyltransferase; PAP, phosphatidic acid phosphatase; DGAT, diacylglycerol acyltransferase; CoA, Coenzyme A; TAG, triacylglycerol, (adapted from [57,59,84]).

TAGs are packed into lipid droplets, but the subcellular localization of these organelles is not fully understood. The lipid droplets are typically located in the cytosol, however, their localization within chloroplast has been under debate [85]. For example, some works have reported detecting lipid droplets in the chloroplast of the *Chlamydomonas reinhardtii* [82,86,87]. But Moriyama and coworkers found no evidence for the presence of lipid droplets within chloroplast in *Chlamydomonas reinhardtii*, rather they were exclusively found in the cytosol [85].

TAGs content is quite different between species of microalgae and is also greatly modulated by cultivation conditions [88]. For example, in the *Coccomyxa subellipsoidea* under nitrogen starvation, the TAGs content increased linearly through 10 days of N starvation from 0.16 % to 12.6 % of dry weight [89]. A high accumulation of neutral lipids, around 92 % of total lipids, was also reported in *Nannochloropsis oceanica* CCALA 978 strain under nitrogen starvation [90]. In most microalgae, TAGs are composed of SFAs and MUFAs with C14–C18 chain length [60,61]. However, the presence of PUFAs, inclusive of very long chain FA (> 20 carbon atoms) into TAGs has also been reported in microalgae in *Nannochloropsis oculata, P. tricornutum and Thalassiosira pseudonana* [60].

1.1.2. Polar lipids

Polar lipids mainly include glycerophospholipids, betaine lipids, glycolipids and sphingolipids which will be described in the following sections. Polar lipids are important structural components in membranes of cells and organelles and are generally quite abundant in microalgae, representing 40–95% of the total amount of lipids [61].

1.1.2.1. Glycerophospholipids or phospholipids

The glycerophospholipids, also called phospholipids (PLs), are composed of a glycerol backbone esterified with one or two fatty acid chains at positions *sn*-1 and *sn*-2 and a phosphate group esterified at position *sn*-3 to which is linked a polar head group [91]. Depending on the polar head group the PLs can be subdivided into 5 major classes: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylglycerol (PG) [91]. Phosphatidic acid (PA), the simplest PL class, does not have a polar head group (**Figure 1.4**) [91]. The PLs with only one FA, are designated as lyso-PLs (*e.g.* lyso-PCs).

The biosynthesis of PLs occurs by the Kennedy pathway, where the precursor of all PLs is PA [59,92]. PA is biosynthesized within plastids through two enzymes glycerol 3-phosphate acyl-transferase and lysophosphatidic acid acyltransferase. PA can be further converted into CDP-DAG, by CDP-DAG synthase, or be converted into DAG, by phosphatidic acid phosphatase. The CDP-DAG is the intermediate of anionic PLs (PI and PG), while DAG is used to form zwitterionic PLs (PC and PE). The biosynthesis of these most complex PLs occurs in the endoplasmic reticulum (via the “eukaryotic” pathway), with exception of PG which is predominantly produced in the chloroplast.

The most abundant PLs classes in microalgae are typically PC and PG which can range from 0.09-37.2 % and 0.15-15.4 %, respectively, depending on the microalga [19,20,93–96]. PCs are mainly located in extra-chloroplast membranes [18] where they participate in the maintenance of the structural integrity of membranes [79]. PGs have been found in significant amounts in thylakoid membranes, where they play important roles in the assembly and stabilization of photosynthetic systems [79,97,98]. Other PL classes reported in the majority of microalgae were PE, PI and Lyso-PLs [31,99–102], but are usually in lower abundance than PC and PG [93]. The PA and PS are scarcely reported and as far as we know were only identified in microalgae *Chlorella kessleri* [96] and *Scenedesmus obliquus* [103].

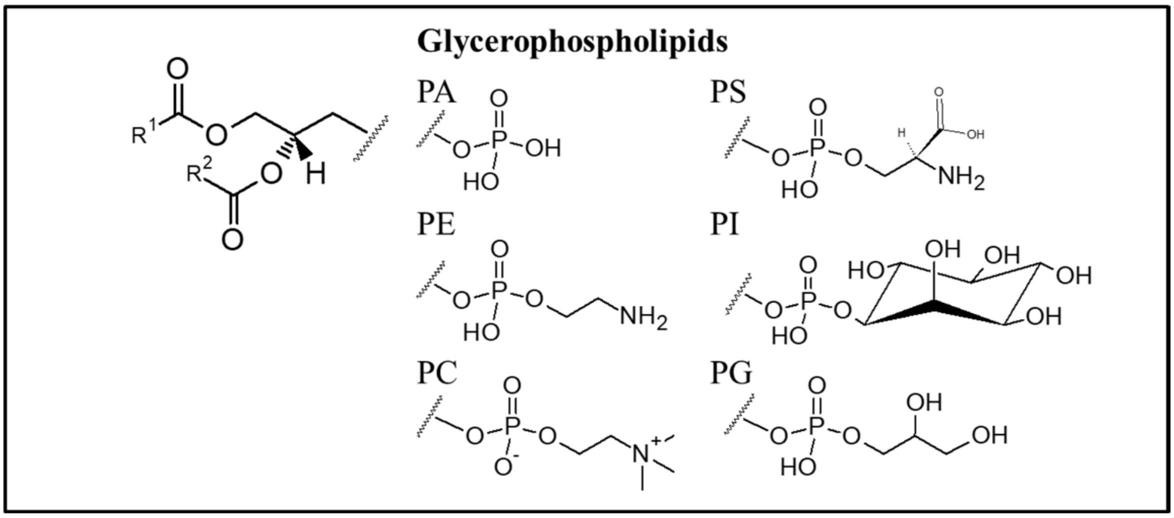

Figure 1.4. Structures of the lipid classes of glycerophospholipids found in microalgae: PA- phosphatidic acid, PC- phosphatidylcholine, PE- phosphatidylethanolamine, PG- phosphatidylglycerol, PI- phosphatidylinositol, PS- phosphatidylserine.

1.1.2.2. Betaine lipids

Betaine lipids have the betaine moiety as a polar group (composed of a positively charged trimethylammonium group) linked to glycerol molecule at position *sn*-3 by an ether bond [104]. They are subdivided into three types: 1,2-diacylglyceryl-3-O-4'-(N,N,N-trimethyl)-

homoserine (DGTS), 1,2-diacylglyceryl-3-O-2'-(hydroxymethyl)-(N,N,N-trimethyl)-β-alanine (DGTA) (structural isomer of DGTS) and 1,2-diacylglyceryl-3-O-carboxy-(hydroxymethyl)-choline (DGCC) [55] (**Figure 1.5**). Their lyso forms, bearing a single FA, are designated as monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine, (MGTS), monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine (MGTA) and monoacylglycerylcarboxyhydroxymethylcholine (MGCC). Its synthesis is thought to occur in ER, *i.e*. via "eukaryotic" pathway. The amount of betaine lipids in microalgae can be quite high, accounting for 0.4-50.7 % of total lipids [19,105]. In terms of intracellular localization, they are mainly found in extraplastidial membranes, but it was also found in membranes of thylakoids in *Chlamydomonas Reinhardtii* [106] and *Isochrysis Galbana Parke* [107]. Regarding its physiological roles, not much is known. It was suggested that they are delivers of FAs to other lipids and can be a substitute lipid of PLs under conditions of phosphorous limitation. The FA composition of betaine lipids from microalgae is mainly comprised of unsaturated FAs and significantly differs from the FA profile of total lipids [61].

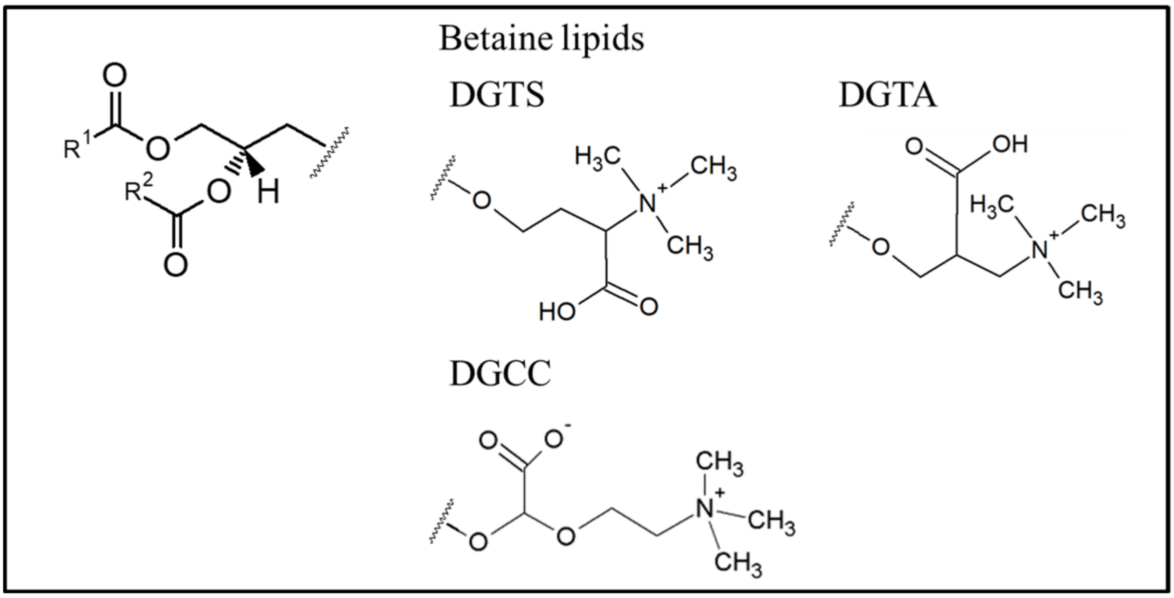

Figure 1.5. Structures of the main lipid classes of betaine lipids found in microalgae: DGTS- diacylglyceryl trimethyl homoserine, DGTA- diacylglyceryl hydroxymethyl-N,N,N-trimethyl-β-alanine, DGCC- diacylglyceryl carboxyhydroxymethylcholine.

1.1.2.3. Glyceroglycolipids or glycolipids

The glyceroglycolipids (or glycolipids) have a glycerol molecule linked to one or two esterified acyl chains at the *sn*-1 and *sn*-2 positions (monoacylglycerol or diacylglycerol) and one or more sugar moieties linked at the *sn*-3 position. Depending on the polar head group and the number of fatty acids (one - monoacylglycerols or two - diacylglycerols), they can be classified as monogalactosylmonoacylglycerol (MGMG), monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), digalactosylmonoacylglycerol (DGMG), sulfoquinovosyldiacylglycerol (SQDG) and sulfoquinovosylmonoacylglycerol (SQMG) (**Figure 1.6**) [29,108,109]. The sugar moieties include galactose residues in the case of neutral glycolipids, DGDG, MGDG and lyso forms or monosaccharides with a sulfonate group, at the 6th position, in the case of acidic glycolipids SQDG, SQMG [57].

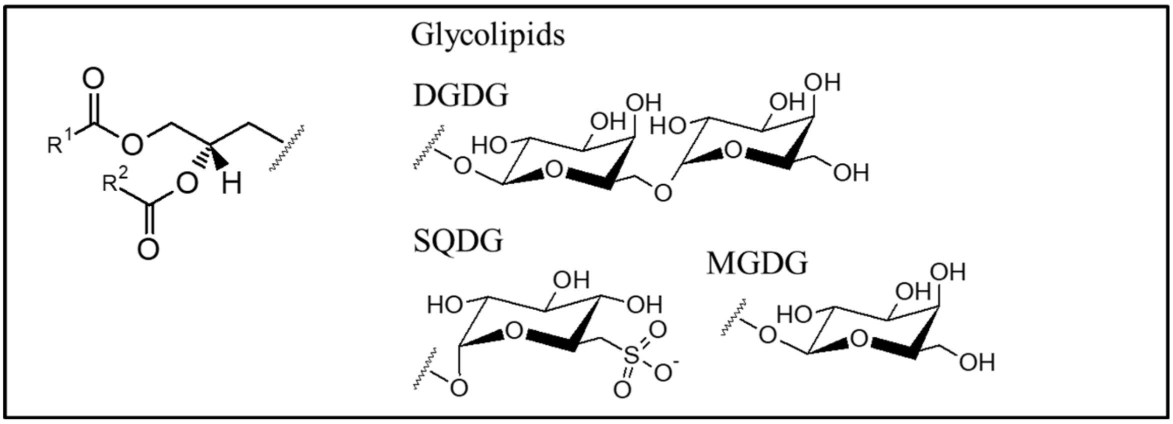

Figure 1.6. Structures of the main lipid classes of glyceroglycolipids (or glycolipids) found in microalgae: DGDG- digalactosyldiacylglycerol, SQDG- sulfoquinovosyldiacylglycerol, MGDG- Monogalactosyldiacylglycerol.

These lipids are present in the chloroplast and thylakoid membranes [57]. With exception of DGDG which can be also found, in a few cases, in extraplastidial membranes [63]. The synthesis of glyceroglycolipids in microalgae is not known but it seems that it occurs in the chloroplast ("prokaryotic pathway"), by the assembly of a glycosidic moiety to DAG [57,110]. DAG can be converted into MGDG, by MGDG synthase and the intermediate uridine 5-diphosphate (UDP) galactose, or be converted into SQDG, by SQDG synthase and the intermediate UDP-sulfoquinovose. DGDG can be further formed through the reaction of MGDG with UDP-galactose and the enzyme DGDG synthase [57,110]. The neutral glycolipids are an important source of PUFAs, act in the stabilization of photosystem subunits and bind to the plastid protein machinery [111]. The SQDGs are negatively charged and are also important in photosynthesis, in chloroplast development [112] and are essential for maintaining the activity and the stability of photosystem II [113]. SQDGs can be replaced by PG in microalgae grown under phosphate-deficient conditions [114].

The most abundant glycolipids in microalgae are typically MGDG ranging from 0.1%–70% of the total lipids, followed by DGDG (0.02%–50%), and SQDG (0.1%–40%) [57]. The FA composition of these glycolipids differs from the glycolipid class, MGDG tends to contain a higher proportion of PUFAs, followed by DGDG and SQDG [59]. MGDG and DGDG are mainly esterified with C16:0, C18:3 *n*-6cis, and C18:2 *n*-6cis FA, while SQDGs are mainly esterified with C16:0, and C18:2 *n*-6cis [61].

1.1.2.4. Sphingolipids

Sphingolipids are constituted by a sphingoid base backbone, instead of glycerol [91], attached to a FA side chain through an amide bond. These *N*-acyl derivatives of sphingoid bases are designated as ceramides (**Figure 1.7**) [115]. More complex sphingolipids such as glycosphingolipids have one or more sugars bounded, via glycosidic linkages, to the primary hydroxyl group of ceramide. Phosphosphingolipids have a phosphate group linked to the primary hydroxyl group of the ceramide. An example of a phosphosphingolipid class is the inositolphosphoceramide (PI-Cer), which contains an inositol group linked to the phosphate group of a ceramide [104]. The most common sphingolipid in mammals, the sphingomyelin with a phosphocholine head group, is not found in microalgae.

The sphingolipids are mainly found in extraplastidial membranes, and their synthesis occurs in the endoplasmic reticulum. The content of sphingolipids in microalgae is typically low and can also vary with the growth conditions. They play structural functions in membranes and act as signalling molecules [116–120].

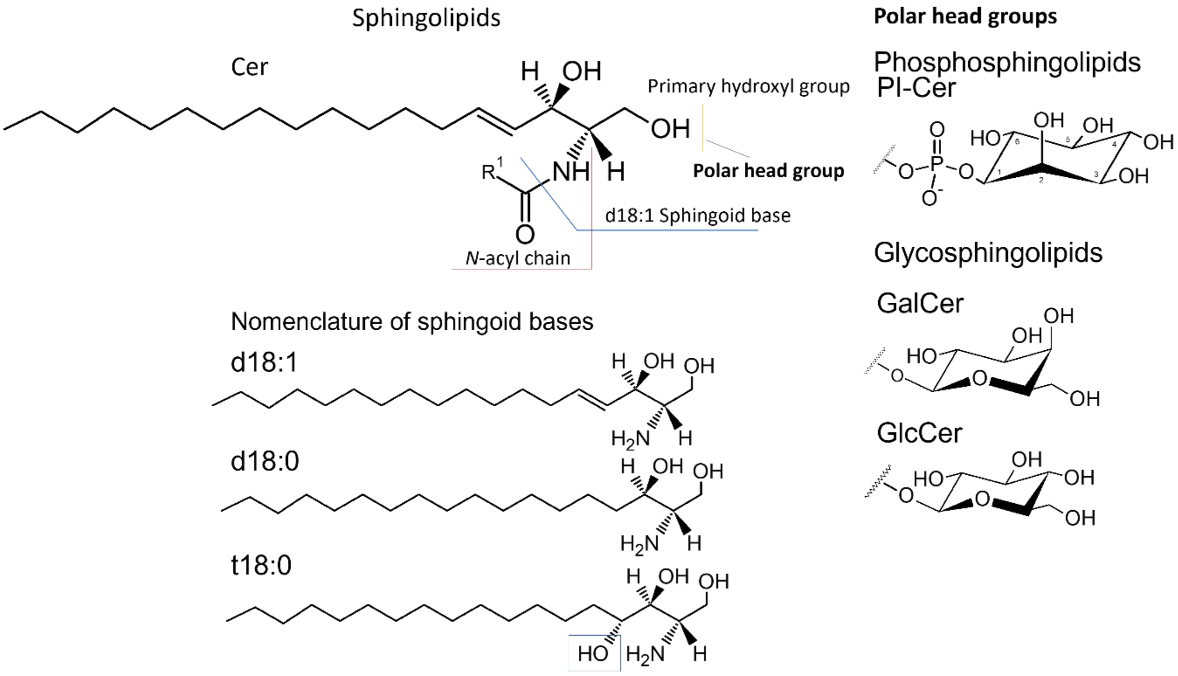

Figure 1.7. Structures of the main sphingolipids found in microalgae. Representation of the Ceramide structure (Cer) with a d18:1 sphingoid base as an example with N-acyl chain and free primary hydroxyl group highlighted in red and yellow squares, respectively. On the right side some examples of polar head groups that can be attached to the primary hydroxyl group of the Cer, which are present in phosphosphingolipids or glycosphingolipids. Examples of phosphosphingolipid class head groups are the phosphate (ceramide-1-phosphate (Cer-1P)) and the inositol phosphate (inositolphosphoceramide (PI-Cer)) groups, examples of glycosphingolipids head groups are the galactose (galactosylceramide (GalCer)) and glucose (glucosylceramide (GlcCer)). Representation of the nomenclature of the most common sphingoid bases: sphinganine ‘‘d18:0’’, sphingosine ‘‘d18:1’’ and phytosphingosine ‘‘t18:0’’, with “d” and “t” representing the two or three hydroxyls groups in sphingoid base structure. The number “18” corresponds to the number of carbons of the sphingoid base, and “1” or “0” corresponds to the number of double bonds of the sphingoid base.

Few studies have described the SL diversity of microalgae, but they reported a great diversity of sphingoid bases with different carbon chain lengths (namely with 18 C and 19 C) and degrees of unsaturation (from 0 to 4). The SL classes mainly found in microalgae were ceramides (Cer), inositolphosphoceramide (PI-Cer) and glycosphingolipids with one, two and three hexosyl moieties [56,77]. SP classes have been found in species of microalgae from almost all phyla, namely in Ochrophyta (*e.g. Phaeodactylum tricornutum* and *Nannochloropsis oceanica*), Haptophyta (*e.g. Isochrysis galbana*, *Pleurochrysis carterae* and *Emiliania huxleyi*), Myzozoa (*e.g. Alexandrium minutum* and *Prorocentrum donghaiense*), Chlorophyta (*e.g. Tetraselmis* sp., *Chlorococcum amblystomatis* and *Chlorella vulgaris*), Rhodophyta (*Galdieria sulphuraria*) and Cyanobacteria (*Arthrospira* *platensis* and *Moorea producens*). However, the sphingolipid profile of thousands of other species belonging to these phyla and microalgae species belonging to phyla Euglenozoa, Cryptista Cercozoa, Glaucophyta and Charophyta remains to be unravelled.

The full lipidome of microalgae that can include hundreds of individual lipid species from different lipid categories (phospholipids, glycolipids and betaine lipids) and different lipid classes can only be addressed using lipidomics approaches based on LC-MS and MS/MS. In the following section, we will describe the main steps of these approaches (lipid extraction, identification, and quantification of the lipid species by MS and MS/MS analysis).

1.2. A brief overview of the lipidomic workflow

The lipidomic approaches based on mass spectrometry (MS) usually coupled to Liquid chromatography (LC-MS) have emerged as a key tool for the molecular characterization and quantification of the lipid molecular species of the lipidome of the microalgae and to decode the alterations in lipid profile that occur in microalgae grown under different growth and environmental conditions [93,95,120–122]. The lipidomic methodology includes several sequential steps and generally begins with lipid extraction from the biological matrix (**Figure 1.8**).

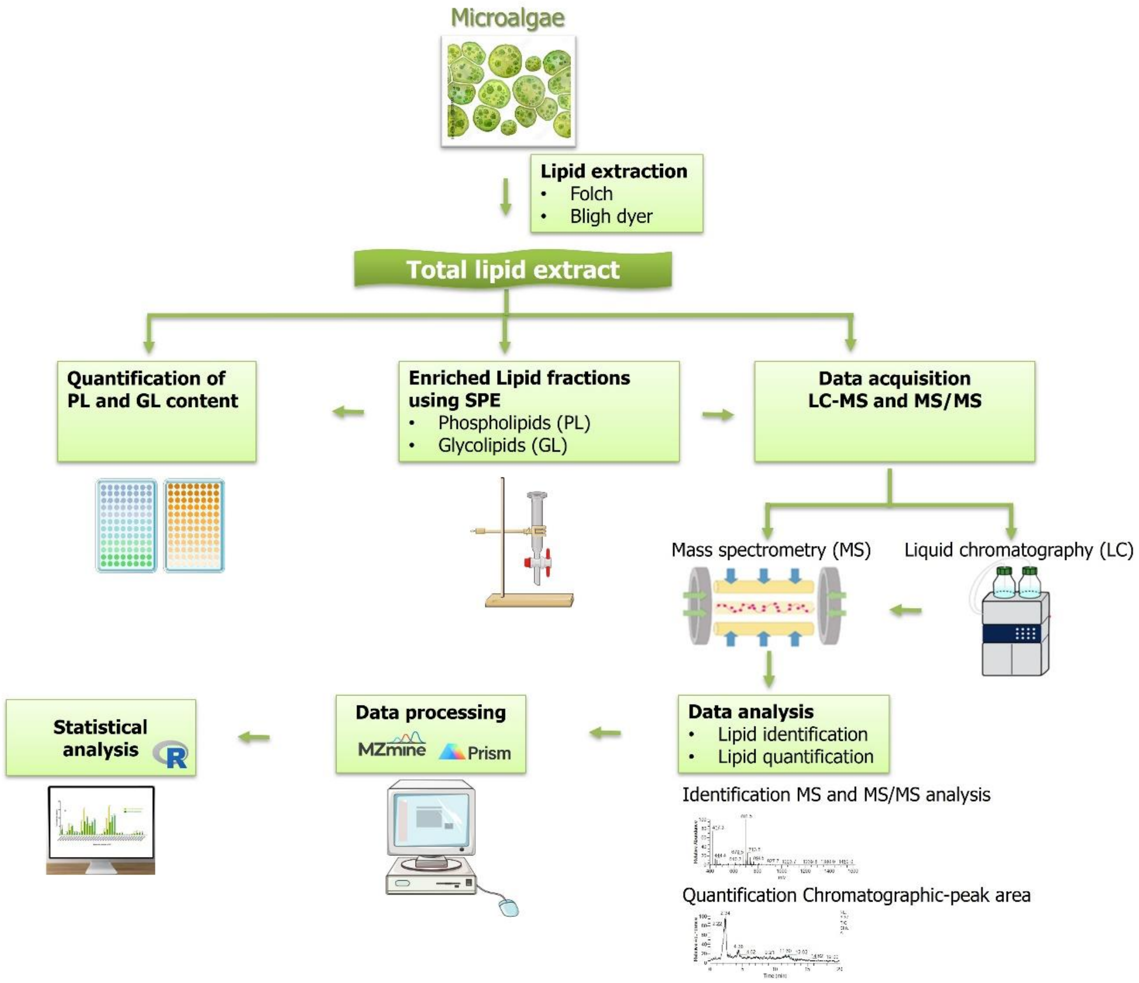

Figure 1.8. The lipidomic workflow.

The mostly used lipid extraction methods are liquid-liquid extraction (LLE) or solid-liquid extraction (SLE). In LLE, different mixtures of organic solvents are used, and the *Blight and Dyer* (chloroform:methanol (1:2 v/v) and *Folch* (chloroform: methanol (2:1 v/v) methods are usually the ones used for the study of lipids in microalgae. More recently, the extraction using MTBE (methyl-tert-butyl ether) (MTBE:MeOH:H_2O (10:3:2.5, v/v/v) was employed to extract lipids from microalgae namely: *Nannochloropsis oceanica* [123], *Cyclotella cryptica*, *Thalassiosira weissflogii*, *Nannochloropsis salina*, *Alexandrium minutum* and *Alexandrium tamutum* [93,124,125]. The use of the MTBE method has the following advantages: the less toxicity of MTBE in comparison to the chlorinated solvents [126], and the localization of lipids in the upper phase, which makes it easier to recover it [127].

Other green solvents have been explored, such as hexane, ethanol, isopropanol, butanol, acetic acid esters, 2-ethoxyethanol. However, lipid extractions carried out with these solvents have typically resulted in lower yields in comparison with traditional methods. To overcome this drawback, new extraction methods have been continually developed (with or without using solvents), such as chemical, physical, enzymatic, and mechanical methods [126]. Examples of these methods are supercritical fluid extraction usually with CO_2, the bead beating method, expeller press, microwave-assisted extraction, pulsed electric field, hydrothermal liquefaction, electroporation or electropermeabilization, osmotic pressure method, isotonic extraction method, enzyme-assisted extraction, pressurized liquid extraction and ultrasound-assisted extractions, as reviewed in [126]. The choice of the methods or the solvents to be used will depend on the polarity of the lipid molecular species of interest (neutral lipids, polar lipids, or the total lipid extract) and also the application of the lipid extract. When the focus of the study is a specific group of lipids the total lipid extract can be fractionated, by solid phase extraction (SPE), in fractions enriched in phospholipids and glycolipids. The amount of phospholipids and glycolipids in the extracts and fractions can be estimated by the molybdovanadate [128] and orcinol [129] methods, respectively.

After extraction, the next step is the analysis of the lipid extract or fractions by mass spectrometry (MS). This analysis can be performed using *targeted* or *untargeted* methodologies [127]. *Targeted* methodologies are used for the analysis of a specific set of lipid species present in the lipid extract. *Untargeted* methodologies consider the analysis of as many lipid species as possible [56]. In both methodologies, the samples can be injected by direct infusion-MS, without previous separation of the lipid species (*shotgun* lipidomics), or using chromatographic techniques coupled to MS for the separation of the lipid species before MS analysis [124]. Liquid chromatographic techniques include several separation methods such as reverse phase (RP), normal phase (NP) or hydrophilic interactions (HILC) [127]. Reverse-phase chromatography allows a selective lipid separation based on the different hydrophobicity of the acyl chain substituents, differences in the length and unsaturation of the fatty acyl chains, and is generally more used to separate different lipid molecular species within the same lipid class [93,130]. The more frequently used columns contain silanol groups derivatised with C8, C18 and C30 chains [124]. Normal phase chromatography and the HILIC column are used for the selective differentiation of the lipids based on the different hydrophilicity, allowing the separation of the lipid classes, but also lipid species, particularly when using HILIC [93,130]. After separation by chromatographic techniques, the structural characterization of the lipids is carried out by MS analysis, as aforementioned. This analysis involves the interpretation of the obtained spectra, based on the molecular ions observed in the MS spectra and the product ions observed in the MS/MS and/or MS^n spectra.

The identification of the lipids in LC-MS is based on the identification of the retention time, the mass accuracy, and the MS/MS analysis. After the characterization of the lipid species, their relative or absolute quantification can be performed. The relative quantification of the lipid species can be achieved by the peak area of the reconstructed ion chromatogram (RIC) of each extracted ion and by the normalization with the peak area of the RIC of specific standards for each lipid class. Data analysis can be supported using bioinformatic tools (such as the mzMine [131], the procedure adopted in this thesis. The absolute quantification, normally requires the use of stable isotope labelled internal standards variants of each lipid class, being recommendable a standard for each lipid species.

1.2.1. Analysis of MS and MS/MS fragmentation pathways of the lipid classes

The lipidome of the microalgae has been identified by the analysis of MS and MS/MS spectra as reviewed in [56,57]. In MS, the molecular ion peak is used to identify the molecular weight of the lipid species. Using accurate mass measurements and comparing these values with the ones on lipid databases is the first step to characterize the lipidome. The ion peak observed in the MS spectra depends on the chemical features of the lipids and ionization efficiency. Positive or negative ion modes can be used, depending on the proton affinity and chemical composition of the polar head group of each lipid class, and also of the eluents used in LC-MS. The typical ions observed are described in **Table 1.2**. For example, PC, PE and betaine lipids are usually identified in the positive mode as $[M+H]^+$, while neutral glycolipids and TAG as $[M+NH_4]^+$. In the negative mode, we can see the PE, PS, PI, PG and SQDG deprotonated $[M-H]^-$ molecular ions.

The MS/MS spectra of each lipid species provide detailed information about the structure of the lipids, including their polar head group and fatty acyl chains. The phosphatidylcholines (PC) and the lysophosphatidylcholine (LPC) can be identified in MS/MS spectra of the $[M+H]^+$ ions with a major product ion at *m/z* 184 corresponding to the phosphocholine polar head group. The MS/MS of the negative ions $[M+CH_3COO]^-$ and $[M+HCOO]^-$ adducts show the product ions resulting from the neutral loss of the 74 Da (loss of methyl acetate, CH_3COOCH_3) and 60 respectively [104], and the ion at *m/z* 168 (which is typical of phosphocholine) [132]. FAs are identified by $RCOO^-$ anion fragment ions.

The PE and LPE are identified in MS/MS spectra of the $[M+H]^+$ ions by the typical neutral loss of 141 Da that corresponds to the phosphoethanolamine moiety ($HPO_4(CH_2)_2NH_3$) and allows for confirming the nature of the polar head [23]. Other characteristic product ions of the polar head group can also be observed in the MS/MS of the $[M-H]^-$ ions at *m/z* 140 (phosphoethanolamine anion, $[C_2H_7NO_4P]^-$) and the ion at *m/z* 196 ($[C_5H_{11}NO_5P]^-$) [132]. The PS, PI and PG are also identified in the MS/MS of the $[M-H]^-$ ions, showing respectively the NL of 87 Da, product ion at *m/z* 241 (inositol phosphate anion) and ion at *m/z* 227 (glycerophosphate glycerol anion - H_2O) [23,132].

In all ESI-CID-MS/MS spectra of the $[M-H]^-$ ions of the phospholipids classes (and of the $[M+HCOO]^-$ or $[M+CH_3COO]^-$ ions of the PC and LPC), is possible to observe the carboxylate anions $[RCOO]^-$ that allow identifying the fatty acyl chains. It is possible to predict the fatty acyl chains that are present at *sn*-1 or *sn*-2 positions of the glycerol backbone, according to the relative abundance of these anions $RCCO^-$, but it can vary, depending on the instrument used, and should always be confirmed by comparison with standards of each PL class [132]. The tendency of the signal intensity $[R_2COO]^-$ and $[R_1COO]^-$ product ions was not always the same among the phospholipids classes [132,133].

The betaine lipids (DGTS, DGTA and DGCC) can be identified as $[M+H]^+$ ions [28,130,134]. The MS/MS spectra of the $[M+H]^+$ ions of the DGTS and DGTA and their lyso-derivatives showed the product ions at *m/z* 236. The relative abundance of these ions indicates the lipid class (DGTS or DGTA), being typically less abundant in DGTA and MGTA species. In the case of the DGCC, the fragmentation via CID of the protonated molecular ions produce characteristic product ions at *m/z* 104 (ions of molecular formula $[C_5H_{14}NO]^+$) [130].

The neutral glycolipids (MGDG, MGMG, DGMG, DGDG) are more prone to ionize in positive ion mode, predominantly through the formation of ammonium $[M+NH_4]^+$ adducts [135,136]. Typical fragmentation of MGMG and MGDG is the NL of NH_3 plus galactosyl unit (-197 Da) [133,137]. In the case of DGMG and DGDG, the NL of NH_3 plus one hexose and one hexose residue (-359 Da) is observed. The acylium $[RCO]^+$ and $[RCO+74]^+$ ions also are observed and confirm the nature of the fatty acyl chains [133,137].

The SQMG and SQDG are more easily ionizable in negative ion mode, with the predominantly formation of $[M-H]^-$ ions. The MS/MS fragmentation of $[M-H]^-$ ions produces the characteristic product ions of their polar head group at *m/z* 225, and the ions formed due to the NL of their fatty acyl chains, as keto and acid derivatives [104,136,138] as well as their carboxylate anions ($RCOO^-$) [23,104,138].

The MS/MS spectra of the $[M+H]^+$ ions of the sphingolipids (Cer and HexCer) show product ions corresponding to the sphingoid base. The most common sphingoid bases in mammals are: d18:1 (at *m/z* 264) and d18:0 (at *m/z* 266) [115]; in plants are: d16:0 (at *m/z* 238), d17:0 (at *m/z* 252), d18:0 (at *m/z* 266), d18:1 (at *m/z* 264), d18:2 (at *m/z* 262), d18:3 (at *m/z* 260), t18:1 (at *m/z* 280), d19:0 (at *m/z* 280), d19:2 (at *m/z* 276), d19:3 (at *m/z* 274), d20:0 (at *m/z* 294), d20:2 (at *m/z* 290) and in microalgae are: d18:0; d18:1; d18:2; d18:3 and d18:4 (at *m/z* 258) [118]. It is important to highlight that these ions do not correspond to the complete structure of the sphingoid base, but instead correspond to the *m/z* value of the sphingoid base minus two water molecules. The MS/MS spectra of the $[M-H]^-$ ions of the PI-Cer show the characteristic product ions at *m/z* 241 corresponding to the inositol-1,2-cyclic phosphate anion.

The MS/MS spectra of the $[M+NH_4]^+$ ions of the neutral lipids (TAG and DAG) show the combined NL of 17 Da (NH_3) and fatty acyl chains as acid derivatives ($RCOOH+NH_3$) [139].

Table 1.2. Typical precursor ions formed in ESI-MS of the main lipid classes identified on lipidome of the microalgae and respective product ions and/or neutral loss produced during the fragmentation of the precursor ions under ESI-MS/MS (Adapted of Ref [104]).

Lipid classes	Precursor ion in MS	Typical product ions and neutral loss in MS/MS	Corresponding to:	Ref
SQDG	$[M-H]^-$	*m/z* 225	Sulfoquinovosyl anion; $[C_6H_9O_7S]^-$	[23,28,36,46,99,137,140]
		m/z 81	$[HSO_3]^-$	[99]
		m/z 207	$[C_6H_7O_6S]^-$	[99]
		m/z 165	$[C_4H_5O_5S]^-$	[99]
	$[M+NH_4]^+$	NL 261 Da	$-C_6H_{15}NO_8S$	[130,141,142]
MGDG	$[M+NH_4]^+$	NL 197 Da (180+17)	$-(Hex_{res}+NH_3)$; $[C_6H_{13}NO_5]$	[123,130,141,142]
DGDG	$[M+NH_4]^+$	NL 359Da (162+180+17)	$-(Hex_{res}Hex+NH_3)$	[133,142]
		NL 341Da (162+162+17)	$-C_{12}H_{23}NO_{10}$; $-(Hex_{res}Hex_{res}+NH_3)$	[123,130,141,142]
Cer; HexCer	$[M+H]^+$	*m/z* 264	Dehydrated sphingosine cation	[104]
DGTS	$[M+H]^+$	*m/z* 236	$[C_{10}H_{22}NO_5]^+$	[23,104,123,134,138,141,143]
		m/z 144	$[C_7H_{14}O_2N]^+$	[28,134]
DGTA	$[M+H]^+$	*m/z* 236	$[C_{10}H_{22}O_5N]^+$	[134]
		m/z 144	$[C_7H_{14}O_2N]^+$	[134]
DGCC	$[M+H]^+$	*m/z* 104	$[C_5H_{14}NO]^+$	[20,130,144]
		m/z 132		[20,144]
PI-Cer	$[M-H]^-$	*m/z* 241	Inositol-1,2-cyclic phosphate anion	[104]
		m/z 259	Inositol monophosphate anion	[104]
PA	$[M-H]^-$	*m/z* 153	Glycerol phosphate anion–H_2O	[104]
	$[M+NH_4]^+$	NL 115 Da	$-[H_6NO_4P]$	[130]
PC, LPC	$[M+H]^+$	*m/z* 184	Phosphocholine cation; $[C_5H_{15}NO_4P]^+$	[23,104,123,130,145]
	$[M+CH_3COO]^-$	NL 74Da	$-CH_3COOCH_3$	[104]
PC	$[M+HCOO]^-$	*m/z* 168	$[C_4H_{11}O_4NP]^-$	[132]

		NL 60Da	-$C_2H_4O_2$ ($HCOOCH_3$)	[132]
PG, LPG	$[M-H]^-$	*m/z* 227	Glycerophosphate glycerol anion -H_2O ($[C_6H_{12}O_7P]^-$)	[21,143]
		m/z 171	Glycerol phosphate anion $[C_3H_7O_2OPO_3H]^-$	[21,143]
		m/z 153	Glycerol phosphate anion - H_2O $[C_3H_6O_5P]^-$	[21,23,143]
		NL 74 Da	-$C_3H_6O_2$, (glycerol as an oxirane)	[143]
	$[M+NH_4]^+$	NL 189 Da	-$C_3H_{12}NO_6P$	[130,141,142]
PE	$[M+H]^+$	NL 141 Da	-($HPO_4(CH_2)_2NH_3$)	[23,123,130,141,142,145]
	$[M-H]^-$	*m/z* 140	Phosphoethanolamine anion $[C_2H_7NO_4P]^-$	[132]
	$[M-H]^-$	*m/z* 196	$[C_5H_{11}NO_5P]^-$	[132]
PI	$[M-H]^-$	*m/z* 241	$[C_6H_{10}O_8P]^-$	[23,132,138,143]
	$[M+NH_4]^+$	NL 277 Da	-$C_6H_{16}NO_9P$	[130,141,145]
PS	$[M+H]^+$	NL 185 Da	-$C_3H_8NO_6P$	[130]
	$[M-H]^-$	NL 87 Da	-(serine+H_2O) ($C_3H_5O_2N$)	[132]
TAG	$[M+NH_4]^+$	NL 17 Da		[139]

As shown in this chapter, the lipidomics workflow includes several complex steps: lipid extraction, fractionation (when necessary), LC-MS acquisition and manual analysis of LC-MS and MS/MS data supported by bioinformatics tools. So far, lipidomics approaches based on LC-MS have been successfully employed in the characterization of the lipid profile of some microalgae species (as reviewed in [56]), with most studies focusing on the lipidome of microalgae belonging to the genus *Chlorella* [19,30,96,142,146], *Nannochloropsis* [19,23,24,30,93,100,101,123,146], *Chlamydomonas* [95,141,146–148] and species of *Phaeodactylum tricornutum* [19,31,94,100].

1.3. State of the art of Lipidomics profile of microalgae

In this chapter, we provide an overview of the microalgae lipidomic studies available in the scientific literature, from 2015 to 2021. The content of this subchapter was reproduced from Rey *et al.*, (2022) [56] with permission from the Royal Society of Chemistry, entitled: "Applications of lipidomics in marine organisms: progress, challenges and future perspectives" to which I am a co-author. This article includes several other topics that are not presented here. This chapter only contains parts of the text of the section designated in the original manuscript as “Lipidomics of macroalgae, microalgae and halophytes”, to which I actively contributed to bibliographic research and the writing of the parts of the section presented here.

Algae (macroalgae and microalgae) are photosynthetic organisms that represent a source of nutritional and functional food with a huge variety of bioactive molecules, including lipids. These features have attracted the interest of the food, cosmeceutical, pharmaceutical, and nutraceutical industries. However, lipidomics studies of both matrices show different development stages. Most of the studies on macroalgae are focused on FA identification and lipidomics studies are scarce, so their lipidome is still in an early disclosure stage. In contrast, microalgae lipids are widely explored representing a large coverage of studies in the marine lipidomics field.

Lipidomics studies of algae have mainly focused on the characterization of lipid signatures, bioprospection, evaluation of lipidomic plasticity under different growth conditions, evaluation of different extraction methodologies, ecotoxicology, and response to global warming, as will be described below.

Studies in microalgae unveiled the lipidome of several species from Chlorophyta [12,146,149,150], Ochrophyta [23,31,93,100,123,150–153], Cyanobacteria [99], Haptophyta [27,154,155], Bigyra [146] and Myzozoa [93] phyla (**Table 1.3**). Most lipidomics studies in microalgae identify GL classes, such as DGDG, MGDG and SQDG; PL classes, including PG, PC, PE, PI; BL classes, including DGTS, DGCC and DGTA; and TG. Although most of these classes are common in the microalgae species studied, some of them, such as PC, PE, and PI, were absent in *Spirulina platensis* [99], *Pavlova gyrans*, *Pleurochrysis carterae* strain CCMP 645, *Isochrysis galbana* strain CCMP 715 and *Phaeocystis antarctica* [154], which could be related to a low abundance of these lipid classes in these algae species or to the MS detection limit of the instrument used in these studies. On the other side, lipid species that are not usually reported in microalgae were identified in *E. huxleyi* [154,155] including MMPE; PDPT, sGSL; hGSL, GlcADG, betaine-like lipids (BLL) and MGCC.

Table 1.3. Lipid classes identified in microalgae, extraction, and identification methods. *Freshwater species.

Algae	Species name	Phylum	Sample origin	Lipid extraction	Compound identification	Lipid classes identified	Lipidomics precision level	Study objective	Reference
Microalgae	*Ceratoneis closterium*	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C_{18} RP-UPLC-ESI-Q-TOF-MS^E	Ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
	Ceratoneis sp.	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C_{18} RP-UPLC-ESI-Q-TOF-MS^E	Ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
	**Conticribra weissflogii*	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C_8 UPLC-(ESI)-Q-TOF-MS^n	DGCC; PC; MGDG; DGDG; SQDG; PG; TG	Lipid species	Growth conditions	Li et al. 2016[20]
		Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C_{18} RP-UPLC-ESI-Q-TOF-MS^E	Ceramides; Monosaccharide ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
	Microchloropsis gaditana	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]; *n*-Heptane; bead milling	GC-FID; C_{18} UPLC-ESI-MS and MS/MS	LPC; PC; DGTS; MGTS; DGDG; DGMG; MGDG; PG; PI; SQDG; SQMG; FFA	Lipid molecular species	Characterization and different extraction procedures	Cauchie et al. 2021[157]
	Amphora sp.	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C_{18} RP-UPLC-ESI-Q-TOF-MS^E	Ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
		Ochrophyta	Aquaculture	$CHCl_3/CH_3OH$ (1:1, *v/v)*	C_{18} RP-UPLC-ESI-Q-TOF-MS^E	DGTS; DGTA	Lipid molecular species	Characterization	Li et al. 2017[134]
	Chaetoceros gracilis	Ochrophyta	Aquaculture	Folch et al. 1957[158]	HPLC-ESI-TOF-MS	DGCC	Lipid molecular species	Characterization	Cañavate et al. 2016[105]
	Cyclotella cryptica	Ochrophyta	Aquaculture	MTBE	UPLC-ESI-MS^n	PC; PE; PI; PG; SQDG; DGDG; MGDG; TG	Lipid molecular species	Characterization	Cutignano et al. 2016[93]
	Cylindrotheca closterium	Ochrophyta	Aquaculture	Modified gravitational method	GC-FID; HPLC-ESI-TOF-MS and	DGDG; MGDG; SQDG; TG	Lipid molecular species	Growth conditions	Wang et al. 2019[150]

				HPLC-ESI-ion-trap-MS^n				
Nannocloropsis oceanica	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C_8 UPLC-(ESI)-Q-TOF-MS	MGDG; DGDG; SQDG; PG; DGTS; TG	Lipid molecular species	Characterization	Li et al. 2015[153]
Nannocloropsis oceanica IMET1	Ochrophyta	Aquaculture	Folch et al. 1957[158]	C_{18} HPLC-ESI-QqQ-MS	DGTS; PC; PE; PI; PG; MGDG; DGDG; SQDG	Lipid molecular species	Growth conditions	Han et al. 2017[23]
Nannocloropsis oceanica	Ochrophyta	Aquaculture	MTBE	HILIC-ESI-MS^n; GC-FID	MGDG; DGDG; SQDG; DGTS; MGTS; PG; PI; PE; PC; LPC	Lipid molecular species	Characterization	Meng et al. 2017[123]
	Ochrophyta	Aquaculture	Folch et al. 1957[158], CH_2Cl_2/CH_3OH CH_2Cl_2/ethanol, ethanol, ethanol assisted by ultrasonic bath, ethanol assisted by ultrasonic probe	HILIC-HR-ESI-MS and MS/MS	SQDG; SQMG; DGTS; MGTS; PG; PI; PE; PC; LPC; PI-Cer	Lipid molecular species	Characterization and different extraction procedures	Melo et al. 2021[151]
Nannochloropsis salina	Ochrophyta	Aquaculture	Folch et al. 1957[158]	LTQ; FT-ICR-MS	DGTS; MGTS; DGDG; MGMG; MGDG; SQDG; PG; DG; TG	Lipid species	Growth conditions	Willetteet al. 2018[24]
	Ochrophyta	Aquaculture	MTBE	UPLC-ESI-MS^n	MGDG; DGDG; SQDG; PG; PI; PE; PC; TG	Lipid molecular species	Characterization	Cutignano et al. 2016[93]
	Ochrophyta	Aquaculture	Folch et al. 1957[158]	GC-MS; LTQ FT-ICR MS	MGTS; DGTS; MGDG; DGDG; SQDG; PG; DG; TG; FFA	Lipid species	Growth conditions	Gill et al. 2018[159]
Nannocloropsis sp.	Ochrophyta	Aquaculture	Folch et al. 1957[158]	Off-line SPE-Si; Off-line TLC; LC-ESI-QqQ-MS^n; GC-MS	DGDG; MGDG; SQDG; PG; LPG; LPC; LPE; PC; PE; PI	Lipid species	Characterization	Yao et al. 2015[146]
Nannochloropsis sp. PJ12	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	GC-MS; C_{18} LC-MS and LC-MS/MS	DGDG; DGTS; LPC; LPE; LPG; MGDG; PA; PC; PE;	Lipid molecular species	Growth conditions	Liang et al. 2019[160]

					PG; PI; SQDG; TG; DG; FFA;			
Nannochloropsis gaditana	Ochrophyta	Aquaculture	Folch et al. 1957[158]	TLC; GC-FID; ESI-LTQ-XL-MS; HPLC-ESI-QqQ-MS^n	SQDG; MGDG; DGDG; PG; PI; PE; PC; DGTS; DG; TG	Lipid molecular species	Characterization and Growth conditions	Jouhet et al. 2017[100]
	Ochrophyta	Aquaculture	Folch et al. 1957[158]	HPLC-ESI-TOF-MS	DGTS	Lipid molecular species	Characterization	Cañavate et al. 2016[105]
Nannochloropsis oculata	Ochrophyta	Aquaculture	CH$_3$OH/CHCl$_3$ (1:1, *v/v*); H$_2$O/ CH$_3$OH/ CHCl$_3$ (3:2:1, *v/v/v*)	(−) ESI FT-ICR MS; NanoLC-FT-ICR-MS; Online Nano LC-MS and MS/MS	DGDG; DGMG; DGTS; PI-Cer; LPC; LPE; MGDG; MGMG; PC; PE; PG; PI; SQDG; FFA	Lipid species	Characterization	Liu et al. 2016[101]
Phaeodactylum tricornutum	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	TLC; GC-FID; ESI-LTQ-MS^n	MGDG; DGDG; SQDG; SQMG; PG; PC; DGTA; PE; PI; DG; TG	Lipid molecular species	Characterization and Growth conditions	Abida et al. 2015[31]
	Ochrophyta	Aquaculture	Folch et al. 1957[158]	TLC; GC-FID; ESI-LTQ-XL-MS; HPLC-ESI-QqQ-MS^n	DGDG; MGDG;DGTA; PE; PC; PG; PI; SQDG; DG; TG	Lipid molecular species	Characterization and Growth conditions	Jouhet et al. 2017[100]
	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C$_{18}$ RP-UPLC-ESI-Q-TOF-MS^E	Ceramides; Disaccharide ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
	Ochrophyta	Aquaculture	Folch et al. 1957[158]	HPLC-ESI-TOF-MS	DGTA	Lipid molecular species	Characterization	Cañavate et al. 2016[105]
	Ochrophyta	Aquaculture	Folch et al. 1957[158]	GC-FID; HPLC-ESI-QqQ-MS and MS/MS	SQDG; MGDG; DGDG; PG; PI; PE; PC; DGTA; DG; TG	Lipid classes	Growth conditions	Jaussaud et al. 2020[152]
Skeletonema sp.	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C$_{18}$ RP-UPLC-ESI-Q-TOF-MS^E	Trisaccharide ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]

Skeletonema tropicum	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C$_{18}$ RP-UPLC-ESI-Q-TOF-MS^E	Trisaccharide ceramides	Lipid molecular species	Characterization	Li, et al. 2017[118]
Skeletonema costatum Skeletonema SKSPXS0711	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C$_{18}$ RP-UPLC-ESI-Q-TOF-MS^E	Disaccharide ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
Skeletonema costatum Skeletonema SCXMBO2	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C$_{18}$ RP-UPLC-ESI-Q-TOF-MS^E	Ceramides; Disaccharide ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
**Stephanodiscus* sp.	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C$_{18}$ RP-UPLC-ESI-Q-TOF-MS^E	Ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
Thalassiosira pseudonana	Ochrophyta	Aquaculture	Bligh & Dyer, 1959[156]	C$_{18}$ RP-UPLC-ESI-Q-TOF-MS^E	Ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
	Ochrophyta	Aquaculture	Folch et al. 1957[158]	HPLC-ESI-TOF-MS	DGCC	Lipid molecular species	Characterization	Cañavate et al. 2016[105]
Thalassiosira weissflogii	Ochrophyta	Aquaculture	MTBE	UPLC-ESI-MS^n	MGDG; DGDG; SQDG; PG; PI; PE; PC; TG	Lipid molecular species	Characterization	Cutignano et al. 2016[93]
**Chlamydomonas reinhardtii*	Chlorophyta	Aquaculture	CH$_3$OH:CHCl$_3$:H$_2$O (5:2:2, *v/v/v*)	Nano ESI-MS^n LTQ	DGTS; MGTS; MGDG; DGDG; SQDG; PG; PI; TG	Lipid molecular species	Growth conditions	Yang et al. 2015[148]
	Chlorophyta	Aquaculture	Folch et al. 1957[158]	Off-line SPE-Si; Off-line TLC; LC-ESI-QqQ-MS^n; GC-MS	DGDG; MGDG; SQDG; PG; LPG; LPE; PC; PE; PI	Lipid species	Characterization	Yao et al. 2015[146]
	Chlorophyta	Aquaculture	Folch et al. 1957[158]	C8 RP-UPLC-ESI-LTQ-Orbitrap-MS	MGDG; DGDG; SQDG; PE; PG; PI; DGTS	Lipid molecular species	Characterization and growth conditions	Yang et al. 2018[141]
	Chlorophyta	Wild	Not applicable	MALDI-MS and MALDI-MS/MS	DGTS, MGDG, DGDG, TG	Lipid species	Ecology (herbicide exposure)	Shanta et al. 2021[161]

	**Chlorella sp.*	Chlorophyta	Aquaculture	Folch et al. 1957[158]	LC-ESI-LTQ-MS/MS; GC-MS	MGDG; DGDG; SQDG; PG; PE; PC; DGTS	Lipid molecular species	Growth conditions	White et al. 2019[142]
	**Chlorella vulgaris*	Chlorophyta	Aquaculture	Folch et al. 1957[158]	HILIC-HR-ESI-MS and MS/MS; GC-MS	PC; LPC; PE; LPE; PG; PI; DGDG; DGMG; MGDG; MGMG; SQDG; Cer; PI-Cer	Lipid molecular species	Growth conditions and Bioprospection	Couto et al. 2021[12]
	**Chlorella vulgaria*	Chlorophyta	Aquaculture	Folch et al. 1957[158]	Off-line SPE-Si; Off-line TLC; LC-ESI-QqQ-MS^n; GC-MS	DGDG; MGDG; SQDG; PG; LPG; LPC; LPE; PC; PE; PI	Lipid species	Characterization	Yao et al. 2015[146]
	**Chlorococcum amblystomatis*	Chlorophyta	Aquaculture	Folch et al. 1957[158]	HILIC-HR-ESI-MS and MS/MS; GC-MS	PC; LPC; PE; LPE; PG; PI; PI-Cer; DGDG; DGMG; MGDG; MGMG; SQDG; SQMG; DGTS; MGTS	Lipid molecular species	Characterization and Bioprospection	Conde et al. 2021[149]
	**Ettlia oleoabundans*	Chlorophyta	Aquaculture	CH$_3$OH assisted by bead milling followed by 2-ethoxyethanol, hexane:toluene:acetone : CH$_3$OH (2:2:1:1, *v/v/v/v*)	LC-ESI-QToF-MS and LC-MS/MS	MGMG; MGDG; DGDG; SQDG; PA; PG; PI; PC; PE; MG; DG; TG; FFA	Lipid species	Growth conditions	Matich et al. 2018[29]
	**Haematococcus pluvialis*	Chlorophyta	Aquaculture	CH$_3$OH/ CHCl$_3$ (1:1, *v/v*); H$_2$O/ CH$_3$OH/ CHCl$_3$ (3:2:1, *v/v/v*)	(−) ESI FT-ICR MS; NanoLC-FT-ICR-MS; Online Nano LC-MS and MS/MS	DGDG; DGMG; DGTS; PI-Cer; LPC; MGDG; PC; PE; PG; PI; SQDG; FFA	Lipid species	Characterization	Liu et al. 2016[101]
	Picochlorum atomus	Chlorophyta	Aquaculture	Folch et al. 1957[158]	HPLC-ESI-TOF-MS	DGTS	Lipid molecular species	Characterization	Cañavate et al. 2016[105]
	Scenedesmus sp.	Chlorophyta	Aquaculture	Folch et al. 1957[158]	Off-line SPE-Si; Off-line	DGDG; MGDG; SQDG; PG;	Lipid species	Characterization	Yao et al. 2015[146]

				TLC; LC-ESI-QqQ-MS^n; GC-MS	LPG; LPC; LPE; PC; PE; PI			
	Chlorophyta	Aquaculture	Modified gravitational method	GC-FID; HPLC-ESI-TOF-MS and HPLC-ESI-ion-trap-MS^n	DGDG; MGDG; DGTS; PG; PE; MGTS; TG	Lipid molecular species	Growth conditions	Wang et al. 2019[150]
Tetraselmis suecica	Chlorophyta	Aquaculture	Folch et al. 1957[158]	HPLC-ESI-TOF-MS	DGTA	Lipid molecular species	Characterization	Cañavate et al. 2016[105]
Emiliania huxleyi RCC1250 (strain AC453)	Haptophyta	Aquaculture	Folch et al. 1957[158]	HILIC-HR-ESI-MS and MS/MS; GC-MS	SQDG; SQMG; DGDG; MGDG; DGMG; MGMG; DGTS; MGTS; DGCC; PC; PE; MMPE; PDPT; PI; PG; LPC; LPE; sGSL; hGSL; Ceramides	Lipid molecular species	Characterization	Aveiro et al. 2020[155]
Emiliania huxleyi (strain CCMP 3268)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole - Orbitrap mass spectrometer	DGDG; GlcADG; MGDG; MGMG; SQDG; PC; PG; BLL; DGCC; MGCC; DGTS; DGTA; PDPT	Lipid species	Growth conditions	Lowenstein et al. 2021[154]
Emiliania huxleyi (strain CCMP 370)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole - Orbitrap mass spectrometer	DGDG; GlcADG; MGDG; MGMG; SQDG; PC; PE; PG; BLL; DGCC; MGCC; DGTS; DGTA; PDPT	Lipid species	Growth conditions	Lowenstein et al. 2021[154]
Emiliania huxleyi (strain CCMP 374)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole -	DGDG; GlcADG; MGDG; MGMG; SQDG; PC; PG; BLL;	Lipid species	Growth conditions	Lowenstein et al. 2021[154]

				Orbitrap mass spectrometer	DGCC; MGCC; DGTS; DGTA; PDPT			
Emiliania huxleyi (strain CCMP 379)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole - Orbitrap mass spectrometer	DGDG; GlcADG; MGDG; MGMG; SQDG; PC; PG; BLL; DGCC; MGCC; DGTS; DGTA; PDPT	Lipid species	Growth conditions	Lowenstein et al. 2021[154]
Emiliania huxleyi (strain CCMP 3266)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole - Orbitrap mass spectrometer	DGDG; GlcADG; MGDG; MGMG; SQDG; PC; PG; BLL; DGCC; MGCC; DGTS; DGTA; PDPT	Lipid species	Growth conditions	Lowenstein et al. 2021[154]
Haptolina ericina (strain CCMP 282)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole - Orbitrap mass spectrometer	DGDG; GlcADG; MGDG; MGMG; SQDG; PC; PE; PG; BLL; DGCC; MGCC; DGTS; DGTA; PDPT	Lipid species	Growth conditions	Lowenstein et al. 2021[154]
Isochrysis galbana (strain CCMP 715)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole - Orbitrap mass spectrometer	DGDG; GlcADG; MGDG; MGMG; SQDG; PG; BLL; DGCC; MGCC; DGTS; DGTA; PDPT	Lipid species	Growth conditions	Lowenstein et al. 2021[154]
Isochrysis galbana	Haptophyta	Aquaculture	CH_3CH_3/CH_3OH (1:1, *v/v)*	TLC; ^1H NMR; SPE-C_{18}; C_{18} RP-UPLC-ESI-MS^n; GC-MS	MGDG; DGDG; GalCer	Lipid molecular species	Characterization and Bioprospection	de los Reyes et al. 2016[49]

	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	C_{18} RP-UPLC-ESI-Q-TOF-MS^E	Monosaccharide ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
Isochrysis galbana Parke	Haptophyta	Aquaculture	$CHCl_3/CH_3OH$ (1:1, *v/v*)	C_8 RP-UPLC-ESI-Q-TOF-MS	DGCC; MGDG; MGCC; MGDG; MGMG; SQDG; SQMG; TG	Lipid molecular species	Growth conditions	Huang et al. 2017[27]
Isochrysis zhanjiangensis	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	C_{18} RP-UPLC-ESI-Q-TOF-MS^E	Monosaccharide ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
Pavlova gyrans (strain CCMP 608)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole - Orbitrap mass spectrometer	DGDG; GlcADG; MGDG; MGMG; SQDG; PG; BLL; DGCC; MGCC; DGTS; DGTA	Lipid species	Growth conditions	Lowenstein et al. 2021[154]
Phaeocystis antarctica (strain CCMP 3314)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole - Orbitrap mass spectrometer	DGDG; GlcADG; MGDG; MGMG; SQDG; PC; PG; BLL; DGCC; MGCC; DGTS; DGTA	Lipid species	Growth conditions	Lowenstein et al. 2021[154]
Phaeocystis globosa (strain CCMP 628)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole - Orbitrap mass spectrometer	DGDG; GlcADG; MGDG; MGMG; SQDG; PC; PE; PG; BLL; DGCC; MGCC; DGTS; DGTA	Lipid species	Growth conditions	Lowenstein et al. 2021[154]
Pleurochrysis carterae (strain CCMP 645)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid	DGDG; GlcADG; MGDG; MGMG; SQDG;	Lipid species	Growth conditions	Lowenstein et al. 2021[154]

				quadrupole - Orbitrap mass spectrometer	PG; BLL; DGCC; MGCC; DGTS; DGTA			
Pleurochrysis carterae	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	C$_{18}$ RP-UPLC-ESI-Q-TOF-MS^E	Monosaccharide ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
*Prymnesium parvum (*strain CCMP 1926)	Haptophyta	Aquaculture	Bligh & Dyer, 1959[156]	Normal and RP-HPLC–Q Exactive hybrid quadrupole - Orbitrap mass spectrometer	DGDG; GlcADG; MGDG; MGMG; SQDG; PC; PE; PG; BLL; DGCC; MGCC; PDPT	Lipid species	Growth conditions	Lowenstein et al. 2021[154]
Alexandrium minutum	Myzozoa	Aquaculture	Bligh & Dyer, 1959[156]	C$_{18}$ RP-UPLC-ESI-Q-TOF-MS^E	Ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
	Myzozoa	Aquaculture	MTBE	UPLC-ESI-MS^n	MGDG; DGDG; SQDG; PG; PI; PE; PC; TG	Lipid molecular species	Characterization	Cutignano et al. 2016[93]
Alexandrium tamutum	Myzozoa	Aquaculture	MTBE	UPLC-ESI-MS^n	MGDG; DGDG; SQDG; PG; PE; PC; TG	Lipid molecular species	Characterization	Cutignano et al. 2016[93]
Gyrodinium dorsum	Myzozoa	Aquaculture	Folch et al. 1957[158]	HPLC-ESI-TOF-MS	DGCC	Lipid molecular species	Characterization	Cañavate et al. 2016[105]

Karlodinium veneficum	Myzozoa	Aquaculture	Bligh & Dyer, 1959[156]	C_{18} RP-UPLC-ESI-Q-TOF-MS^E	Ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
Prorocentrum donghaiense	Myzozoa	Aquaculture	Bligh & Dyer, 1959[156]	C_{18} RP-UPLC-ESI-Q-TOF-MS^E	Ceramides; Monosaccharide ceramides	Lipid molecular species	Characterization	Li et al. 2017[118]
Chroomonas placoidea	Cryptophyta	Aquaculture	Folch et al. 1957[158]	HPLC-ESI-TOF-MS	DGTA	Lipid molecular species	Characterization	Cañavate et al. 2016[105]
Rhodomonas baltica	Cryptophyta	Aquaculture	Folch et al. 1957[158]	HPLC-ESI-TOF-MS	DGTA	Lipid molecular species	Characterization	Cañavate et al. 2016[105]
Spirulina platensis	Cyanobacteria	NA	$CH_3OH/CHCl_3$ (1:1, *v/v*); $H_2O/$ $CH_3OH/$ $CHCl_3$ (3:2:1, *v/v/v*)	RP-UPLC-HRMS (HESI-Q Exactive hybrid quadrupole-Orbitrap mass spectrometer C_{18}	LPG; PG; PI; SQMG; SQDG; MGMG; MGDG; DGMG; DGDG	Lipid molecular species	Other	La Barbera, et al. 2018[99]
Schizochytrium limacinum	Bigyra	Aquaculture	Folch et al. 1957[158]	Off-line SPE-Si; Off-line TLC; LC-ESI-QqQ-MS^n; GC-MS	DGDG; MGDG; SQDG; PG; LPG; LPC; LPE; PC; PE; PI	Lipid species	Characterization	Yao et al. 2015[146]

The diversity of lipid classes found using lipidomic approaches depends on the objective of the study. The first lipidomic survey in a new species usually performs an in-depth characterization of its lipid classes and molecular species. However, specific lipid classes have been the focus of some studies [105,118,134], including the BL (DGTS, DGTA and DGCC). The biological relevance of this group of lipids has slowly been recognized since its first reports in the early seventies, and there is a need to expand BL research from algae. The amount of DGTS, DGTA and DGCC, was identified as a factor to differentiate several microalgae species [105]. DGTS is the most common BL in microalgae, while DGCC has been mainly found in species from Haptophyta phylum. Improving the lipid species identification process has been a concern in lipidomic studies. The optimization of chromatographic conditions and the use of online platforms have been some of the advances in this research area. A good example is the use of Lipostar platform, which improved the identification of phosphoglycerolipids and allowed the identification of glycosylmonorayl- and glycosyldiradyl-glycerols classes [99]. Also, an UPLC-Q-TOF MS method with MS^E data collection mode was developed as an effective analytical tool for the identification of sphingolipids (ceramides, monosaccharide-, disaccharide- and trisaccharide ceramides) from seventeen strains of microalgae from Bacillariophyta, Dinophyta and Haptophyta phyla [118].

Lipidome characterization studies are useful to establish a lipid identity for each alga species [162] which can be used as a chemotaxonomic tool for the differentiation of two identical morphological strains of *Nannochloropsis oceanica* (NMBluh014 and NMBluh-X) [153].

Studies of algae lipidome characterization have provided the nutritional value of these organisms as sources of bioactive PL and GL. The PL and GL are esterified to a high proportion of *n*-3 (omega-3) and *n*-6 (omega-6) PUFA (*e.g.*, [149,162,163]), fostering the use of algae as food resources, because *n*-3 PUFA bound to PL and GL are more bioavailable than PUFA bound to TG [164]. Additionally, some polar lipids have intrinsic bioactive properties being recommended as food ingredients with health-promoting proprieties [165].

In the bioprospecting field, several bioactivities have been associated with macroalga [166,167] and microalga [168] lipids. Some studies have evaluated the bioactivity of lipid extracts from macro and microalgae [169,170] without lipid characterization. On the other hand, total lipid extracts of algae have been evaluated using antioxidant

[12,149,151,163,171–174], anti-inflammatory [49,53,55,167,173,175–177], antiproliferative [167], anti-tumour [45], antiatherogenic [37], anti-viral [178–181] assays followed by characterization of the bioactive extract by LC-MS or nuclear magnetic resonance (NMR). These studies identified molecular species of GL, PL, BL and galactosylceramides as bioactive compounds. However other lipid-soluble molecules (*e.g.*, photosynthetic pigments) present in bioactive extracts could contribute individually or in synergy for the recorded bioactivity. The bioactivity of lipids from algae is a recent research field that needs further exploration since there are few studies that have isolated and characterized the bioactive species [53,55,176,177,182–184]. The current development in the omics fields could boost this area of study, namely disclosing the structure-activity relationship of lipid species.

Several studies have analysed the lipidome plasticity of algae under different growing conditions. Studies performed on microalgae evaluated lipidome plasticity under depletion or repletion of nutrients [23,29,31,100,141,142,148,150,152,154,160], autotrophy or heterotrophy [12], static and aerated culture conditions [20], temperature changes [24,27,159] and light stress [24]. The effect of growing conditions was evaluated on *C. vulgaris*, concluding that if it grew under autotrophic conditions its lipidome present a high content of GL and polar lipid species esterified with *n*-3 FA, while when it was grown under heterotrophic conditions its lipidome was rich in PL and lipid species with *n*-6 FA [12]. Another study on *Nannochloropsis gaditana* verified that high light-induced accumulation of DGTS and TG and decrease of chloroplast polar lipids (MGDG) [185]. Indeed, a similar tendency (*e.g.*, MGDG reduction and TG accumulation) was reported for *N. oceanica* IMET1 under high light exposure, but with an opposite trend in the case of extraplastidic lipids (DGTS, PC) [23]. A reduction in MGDG has also been reported in microalgae species growing at low temperatures [159] and phosphorus depletion [160]. Increase of the DGTS content (phosphorus-free BL) is expected in algae growing under phosphorus depletion [160,186]. Other studies verified the variation in lipid molecular species, mainly in the TG profile under nitrogen starvation [141,150] although with a different response. *Scenedesmus* sp. and *Chlamydomonas reinhardtii* species synthesized TG via the eukaryotic pathway [141,150], while *Cylindrotheca closterium* preferentially used the prokaryotic pathway [150]. The different response under external conditions activates different mechanisms of lipid synthesis and accumulation according to microalga species. This knowledge is very important in selecting candidates for mass production of biofuels or healthy lipids for nutraceutical purposes.

Lipidomics has also been applied to find alternative extraction procedures using food-grade solvents and sustainable methodologies to obtain lipid extracts compatible with food applications [151,171]. In the case of *N. oceanica* and *C. vulgaris* the authors reported that lipid extracts obtained using ethanol and assisted with ultrasound had a lipid composition similar to those obtained using conventional methods based on Folch extraction protocol [151,158,171]. The authors of these studies suggested an alternative green lipid extraction method to provide safe extracts for functional food applications.

An ecotoxicological study to evaluate the effect of herbicides (atrazine, clomazone, and norflurazon) on the microalga *Chlamydomonas reinhardtii* was performed using microchip technology in combination with matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) for lipidomic profiling [161]. The authors verified a decrease of DGDG and DGTS lipid species and an increase of TG in alga lipidome after exposure to herbicides.

Until the beginning of the research studies that are part of the present thesis, the lipidomic approaches based on mass spectrometry have allowed identifying and quantifying the lipid composition of several microalgae, as previously described, and summarized in **Table 1.3**. Nevertheless, in most studies, the authors have identified a few lipid species, perhaps because of the lower sensitivity of the method used or because the authors only wanted to analyze a specific group of lipids. Only the most recent articles, using more sensitive instruments, for example, high-resolution analyzers such as the orbitrap, have allowed identifying a higher number of lipid species, so there is still a lot of work to do in this area of research.

The identification of lipidome is especially important for microalgae with commercial application, to know their nutritional and pharmacological properties and to explore and value these microalgae as a source of high-value lipids with bioactive proprieties and thus to explore new applications. The bioactive properties of microalgal lipids have been evaluated by some authors [13,39,42,151,187]. So, in the following subchapter 5, we briefly described the current studies that reported the potential antioxidant and anti-inflammatory properties of the lipids from microalgae.

1.4. Biological activities of lipids from microalgae

Microalgae are a source of several bioactive compounds and some publications have highlighted the bioactive properties of lipids obtained from microalgae. The existence of thousands of microalgae species, each one possessing a specific lipid profile, makes the bioprospection of the microalgae lipids a great challenge but it also represents an opportunity for the development of new biotechnological applications. Nowadays lipid extracts, lipid fractions and lipid species from microalgae have been evaluated in terms of their possible biological activities, including anti-tumour [44–47], antioxidant [13,39,40], anti-inflammatory [49–55], antimicrobial (antibacterial and antifungal) [41–43], and others [36–38,46,188,189].

Among the reported biological activities of the lipids of microalgae, the antioxidant and anti-inflammatory activities will be the main focus of this thesis. Antioxidant compounds from natural sources have caught the interest of researchers as they are safer than synthetic ones and have several applications in the food, nutraceutical, pharmaceutical and cosmetic industries [190]. Antioxidant proprieties have been reported for lipid extracts of microalgae, by their radical scavenging activity against DPPH and ABTS radicals [13,39,40]. Only extracts of ten microalgae were surveyed: *Scenedesmus intermedius* [42], *Gloeothece* sp. [39], *Chlorella vulgaris* [187], *Chrysotila pseudoroscoffensis* [40], *Chlorococcum amblystomatis* [13], *Scenedesmus obliquus* [13], *Tetraselmis chuii* [13], *Phaeodactylum tricornutum* [13], *Spirulina* sp. [13], and *Nannochloropsis oceanica* [13,151]. The knowledge of the antioxidant properties of microalgal lipids will enhance the value of microalgae since antioxidants protect the human body against oxidative stress [191]. Oxidative stress is known to be involved in the development of several chronic diseases (such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer) and can lead to inflammation [192].

Regarding the anti-inflammatory potential of microalgal lipids, eleven microalgae species were evaluated (*Isochrysis galbana* [49,52], *Tetraselmis chuii* [53], *Chlorella sorokiniana* [54], *Chlamydomonas debaryana* [50,51], *Nannochloropsis gaditana* [50,51], *Nannochloropsis granulata* [55,176], *Oxyrrhis marina* [193], *ETS-05 cyanobacterium*

[194], *Porphyridium cruentum* [189], *Porphyridium aerugineum* [177] and *Pavlova lutheri* [169]). The lipids identified with bioactivity were MGDG, DGDG and DGTS rich fractions, and the following individual lipids species: [DGDG (18:5/18:4; 18:5/16:1, 20:5/16:0, 20:5/16:1, 20:5/14:0 and 20:5/20:5], [MGDG (18:4/16:4; 18:3/16:4, 20:5/16:0, 20:5/16:1, 20:5/14:0 and 20:5/20:5], [MGMG (16:2; 16:3)], SQDG (22:6/16:0), [DGTS (20:5/20:5, 20:5/20:4, 20:5/14:0, 20:5/16:0, 20:5/16:1, 20:5/18:2] and 4 oxylipins. The anti-inflammatory activity of microalgae lipids has been mostly evaluated for their capacity to reduce the levels of pro-inflammatory cytokines (interleukin-1β (IL-1β), IL-6, tumour necrosis factor α (TNFα), etc.), pro-inflammatory mediators (such as nitric oxide (NO)), and the expression of pro-inflammatory enzymes (such as cyclooxygenase-2 (COX-2) and iNOS) in LPS-stimulated macrophages.

Inflammation and cancer are closely related, thus lipids with anti-tumour activity may be an indicator of possible anti-inflammatory potential. Only a couple of studies have addressed the potential anti-tumour activity of lipids of SQDG, MGDG and DGDG esterified with the most common FAs found in microalgae (14, 16 and 18 carbon atoms) [44–48,189]. Only lipids from 5 species of microalgae were surveyed (*Thalassiosira weissflogii* [47], *Porphyridium cruentum* [189], *Phaeodactylum tricornutum* [44], *Phormidium tenue* [48], *Chlorella vulgaris* [45]) and 27 cyanobacteria strains [46].

Overall, although some studies have been published on the bioactivity of microalgae, it is far from being known the biological activities of lipids from microalgae and their possible applications. Moreover, there is a lack of knowledge of the structure-activity relationship of the lipids, and how these lipids modulate (activate or inhibit) the signalling pathways. More studies should be performed to fully understand the true biological activities of these compounds with high added value with potential for applications in diverse industries.

1.5. Thesis objective and structure

The lack of knowledge about the lipidome of microalgae is currently a gap that prevents researchers from unravelling the true biotechnological potential of these remarkable organisms. This lack of knowledge has encouraged the development of this thesis, which aims to describe and catalogue the total lipidome of microalgae, allowing the identification of new bioactive lipids and the study of alterations in the lipid profile in different growth conditions. Microalgae produced in aquaculture are easy to be cultivated under controlled conditions that can be manipulated to enhance the production of target bioactive compounds. Since microalgae are much less vulnerable to contaminants, they are therefore safer for human and animal consumption.

With this in mind, the objectives of this thesis were to:

- Evaluate the differences in the polar lipid signature of two novel *Chlorella vulgaris* mutants (C-Honey and C-White), using high-resolution C18 LC-MS analysis, and determine the potential antioxidant and anti-inflammatory activities of lipid extracts by *in chemico* assays.
- Evaluate the chemoplasticity of the polar lipid profile of *Chlorella vulgaris* grown under autotrophic (C-Auto) and heterotrophic (C-Hetero) conditions, using high-resolution HILIC-LC-MS analysis, and determine the potential antioxidant and anti-inflammatory activities of the lipid extracts by *in chemico* assays.
- Evaluate the lipid extraction efficiency of food-grade ethanol, ultrasound-assisted ethanol (UAE) and dichloromethane/methanol (DCM) of C-Auto and C-Hetero and compare the lipidome composition, by HILIC-LC-MS analysis, and determine the potential antioxidant activity of the lipid extracts by *in chemico* assays.
- Evaluate the changes in the lipid profile of a marine species (*N. oceanica*) and a freshwater species (*N. limnetica*) of *Nannochloropsis* grown in indoor and outdoor photobioreactors, by HILIC-LC-MS analysis, and determine the potential antioxidant activity of the lipid extracts by *in chemico* assays.
- Identify and compare the polar lipidome of a taxonomically diverse set of 7 species of Chlorophyta (*Chlorella. vulgaris*, *Scenedesmus obliquus*, *Tetraselmis chuii*, *Chlorococcum amblystomatis*), Ochrophyta (*Nannochloropsis oceanica*, *Phaeodactylum tricornutum*) and Cyanobacteria (*Spirulina* sp.) phylum, using high-

resolution HILIC-LC-MS analysis, to assess the chemotaxonomic specificity of polar lipids and their potential as sources of valuable ingredients.

- Evaluate the anti-inflammatory potential of lipid extracts and lipid fractions enriched in phospholipids and glycolipids from *N. Oceanica* in LPS-stimulated RAW 264.7 macrophages.

This thesis contributes to the exploitation and valorisation of microalgae, unravelling their biotechnological potential as a source of nutraceuticals and food supplements and creating the opportunity for new products and applications with high added value, in accordance with the 2030 Agenda goals and strategy. This work has been integrated into the project "*Algavalor-Microalgas: produção integrada e valorização da biomassa e das suas diversas aplicações*", funded by Portugal 2020 which was launched due to the collaboration of the University of Aveiro and a Portuguese biotechnology industry (CMP-Microalgae Production Unit).

The results obtained during the work reported in this Ph.D. thesis are presented over 7 chapters. Chapter 2 describes the study performed to evaluate the similarities and dissimilarities between the polar lipid profile of the *Chlorella vulgaris* grown under heterotrophic (C-Hetero) and autotrophic conditions (C-Auto). In this study, we also described the antioxidant and anti-inflammatory activities of the lipid extracts of both *C. vulgaris*. Chapter 3 reports the comparison of the lipid profile of lipid extracts of C-Auto and C-Hetero extracted with a solvent and technology allowed in the food industry (ethanol and ethanol assisted by ultrasound probe), and how it compares with traditional extraction methods. The resulting lipid extracts were also screened for their antioxidant activity. Chapter 4 describes the similarities and dissimilarities between the polar lipid profile of the two new *C. vulgaris* strains developed by chemically-induced random mutagenesis, and cultured heterotrophically. We also evaluated the antioxidant and anti-inflammatory activities of both *C. vulgaris* lipid extracts. Chapter 5 describes the study performed to evaluate the polar lipid profile of the two species of *Nannochloropsis* (*N. oceanica* and *N. limnetica*) and the lipid plasticity of both *Nannochloropsis* species grown indoors and outdoors. All lipid extracts were also screened for their antioxidant activity. Chapter 6 evaluated the chemotaxonomic potential of the microalgae polar lipids, comparing the polar lipidome of 7 species of microalgae belonging to 3 different phyla, Chlorophyta (*C. vulgaris, S. obliquus, T. chuii, C. amblystomatis*), Ochrophyta (*N. oceanica, P. tricornutum*) and

Cyanobacteria (*Spirulina* sp). Chapter 7 describes the anti-inflammatory potential of lipid extracts and lipid fractions from *N. Oceanica* in LPS-stimulated macrophages. Finally, Chapter 8, discusses the main findings of this Ph.D. thesis and future research will be proposed.

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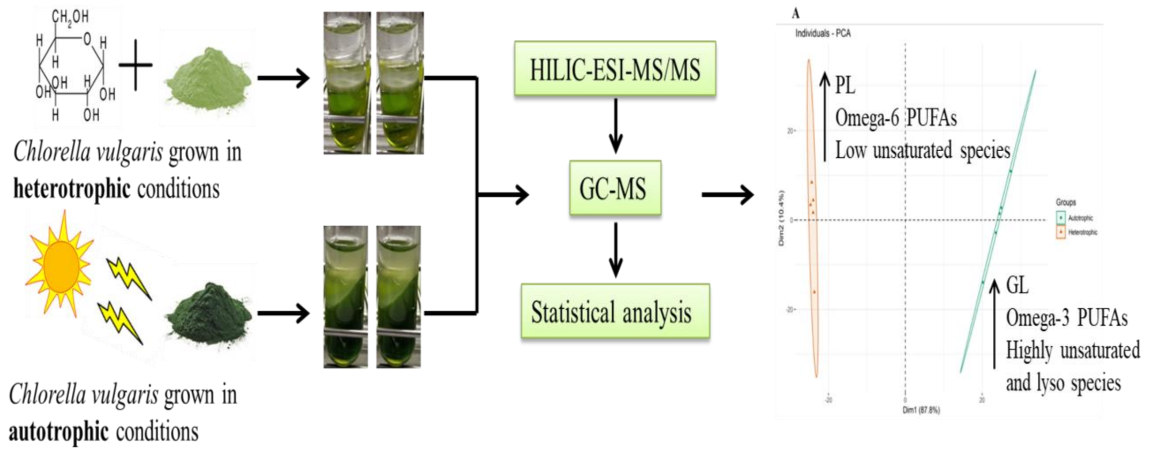
CHAPTER 2 - Chemoplasticity of the polar lipid profile of the microalgae *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions



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Chemoplasticity of the polar lipid profile of the microalgae *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions

Abstract: Chlorella growing under different conditions can adapt its biochemical composition and its fatty acid profile, but the alterations in polar lipids remain poorly explored. In this study, the polar lipidome of *Chlorella vulgaris* grown under autotrophic (C-Auto) and heterotrophic (C-Hetero) conditions were characterized using hydrophilic interaction liquid chromatography–electrospray ionization mass spectrometry and gas chromatography–mass spectrometry. A total of 173 and 167 lipid species were identified in autotrophic and heterotrophic cultures, respectively, distributed by lyso and phosphatidylcholine, lyso and phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, sulfoquinovosyldiacylglycerol, di- and monogalactosyldiacylglycerol, di- and monogalactosylmonoacylglycerol, ceramide, and inositolphosphoceramide lipid classes. C-Hetero had a higher abundance of low unsaturated species, while C-Auto had a high abundance of highly unsaturated glycolipids and lyso species. The fatty acids profile showed a high content of omega-3 polyunsaturated fatty acids in C-Auto and in omega-6 fatty acids in C-Hetero. The two lipid extracts showed high antioxidant activity and inhibitory capacity for cyclooxygenase-2. This study provides evidence of polar lipid plasticity of microalgae useful for tuning cultivation for biotechnology applications.

Keywords: microalgae, *Chlorella vulgaris*, lipidomics, polar lipids, heterotrophy, autotrophy

1. Introduction

Microalgae are a renewable and sustainable source of high-value bioactive compounds, making them valuable ingredients for various industrial applications [1]. Among those, lipids are gaining considerable interest since several microalgae can produce large quantities of lipids. Some of these species produce a high content of omega-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, which are associated to health benefits, including a reduced risk of cardiovascular events and coronary mortality [2]. Also, the total lipid content of microalgae can be high, representing 1% to 90% of the biomass of microalgae [3]. However, the total lipid content and the composition of microalgae depend on the species and growth conditions [4]. The chemo-plasticity of microalgae under different growth conditions can be an advantage because the manipulation of the growth conditions can improve the production of lipids of interest [4].

Chlorella is an edible microalga and is one of the most consumed microalgae in the world, with a market value estimated at billions of dollars [5]. Nowadays, it is used in several biotechnological applications such as cosmeceutical, nutraceutical and pharmaceutical, either as a whole or by using its extracted compounds, in combination or alone, with other ingredients. *Chlorella* is “Generally Recognized As Safe” (GRAS) according to the Center for Food Safety and Applied Nutrition, promoting its use as a dietary supplement and in animal feed [5]. Also, it has been accepted by the European Novel Food Regulation and American Food Drug Administration for the development of functional foods and nutraceuticals [6]. It is generally produced under autotrophic conditions [7], but in recent years, several publications have described the growth of *Chlorella* in other conditions, in particular in heterotrophy [8–15]. This growth is more profitable than traditional culture because of its lower production costs. Furthermore, the cultures are grown in closed systems with a tightly controlled environment allowing easier manipulation to produce biomass of constant composition, improving the productivity and being less prone to contamination by microorganisms [16]. Furthermore, the results of some previously published works have shown that heterotrophic culture conditions are also associated with higher growth rate and lipid production [17], often considered desirable, for example, for production of third-generation biofuels [10].

Chlorella growing under heterotrophic conditions has already been monitored for its protein [9–11], amino acid [11], carbohydrate [18] and lipid [12–14,18] content. Studies reporting lipid composition and plasticity have focused on the variation of the fatty acids (FAs) profile [12,13,18–20]. The fatty acids profile under these two conditions has already been compared for several *Chlorella* species, namely *Chlorella saccharophila* [13], *Chlorella homosphaera* [12], *Chlorella* sp [12,18] and *Chlorella minutissima* [12], and other microalgae species such as *Nannochloropsis salina* [20] and *Galdieria* sp. [19].

The comparison between the polar lipidome of microalgae between autotrophic and heterotrophic conditions is still unknown. Polar lipids play crucial biological functions in cell signaling pathways [21], in the integrity of the structure and fluidity of the membrane and in the response to changes in the cellular environment [21]. These lipids have emulsifying properties [2] and have been associated to bioactive properties, such as antioxidant [22], antimicrobial [23–25], antitumor [26–30] and anti-inflammatory activity [6,31,32]. These polar lipid bioactivities increase the added value of microalgae and contribute to their valorization and will stimulate the emergence of new applications for microalgae biomass.

The polar lipidome under autotrophic conditions of *Kyo*-*Chlorella* [33], *Chlorella vulgaris* [34], *Chlorella kessleri* [35] and *Chlorella* sp. [36–38] was already characterized. For *Chlorella* sp. changes in the profile of polar lipids during all growth phases have been reported [36], but also in cases of nitrogen starvation [37] and nutrient limitation [38]. Therefore, to increase knowledge on the chemoplasticity profile of polar lipids of microalgae cultivated under different conditions, the objective of this work was to evaluate the plasticity of the polar lipidome of *Chlorella vulgaris* grown auto- (C-Auto) and heterotrophic (C-Hetero) conditions. The characterization of the polar lipid and the fatty acids profile of the microalgae was carried out by hydrophilic interaction liquid chromatography (HILIC) electrospray ionization (ESI) mass spectrometry (MS), tandem MS (MS/MS) and gas chromatography-mass spectrometry (GC-MS). In addition, to improve the exploitation and valorization of *Chlorella vulgaris*, we evaluated the bioactive potential of the two lipid extracts by measuring the cyclooxygenase-2 (COX-2) inhibition, and the antioxidant properties using the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS) and α,α-diphenyl-β-picrylhydrazyl radical (DPPH) assays.

2. Materials and methods

2.1. Microalgae strain and culture media

Chlorella vulgaris 0002CA was obtained from the Allmicroalgae culture collection. The heterotrophic inoculum of *C. vulgaris* was stored cryopreserved in liquid nitrogen and was collected and transferred to 50 mL Erlenmeyer flasks when necessary.

2.1.1. Autotrophic culture

The cultures were grown autotrophically in Guillard's F2 culture medium as described previously [39]. Briefly, 5 L flask reactors were cultured for 7 to 15 days, under continuous exposure to light. Twenty 5 L flask reactors were used to inoculate a 1 m^3 L Flat Panel (FP) reactor, which was then used as *inoculum* of a 10 m^3 photobioreactor (PBR). From 10 m^3, the culture was sequentially scaled up to 35 and later 70 m^3 PBRs.

The FPs were kept in a greenhouse at controlled temperature and radiation and the PBRs were kept outdoors, in the facilities of the Allmicroalgae production plant (Fábrica Cibra Pataias, 2445-287 Pataias, Portugal). The pH was kept constant by pulse injections of CO_2.

2.1.2. Heterotrophic culture

The cultures were grown heterotrophically using a C: N ratio of 6.7:1 and glucose (Sapecquimica, Vila Nova de Gaia, Portugal) as the source of organic carbon, as previously described [8]. The heterotrophic culture was obtained sequentially from 50 to 250 mL Erlenmeyer's to 5 L bench-top fermenter (New Brunswick BioFlo® CelliGen®115; Eppendorf AG, Hamburg, Germany), and later, to industrial fermenters of 200 L and 5000 L. All fermenters were operated in fed-batch under controlled temperature, pH and dissolved oxygen.

2.2. Reagents

High performance liquid chromatography (HPLC) grade dichloromethane (CH_2Cl_2), absolute ethanol and methanol (MeOH) were purchased from Fisher Scientific Ltd. (Loughborough, UK). All other reagents have been purchased from major commercial sources. Milli-Q water was obtained from a water purification system (Synergy, Millipore Corporation, Billerica, MA, USA). Phospholipid internal standards 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dimyristoyl-*sn*-glycero-3-phospho-(10-rac-glycerol), 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylinositol, N-palmitoyl-D-*erythro*-sphingosylphosphorylcholine, 1-nonadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). The α,α-diphenyl-β-picrylhydrazyl radical ($DPPH^{\bullet}$) was purchased from Sigma-Aldrich (St Louis, MO, USA) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet +}$) was obtained from Fluka (Buchs, Switzerland). Ammonium acetate, dimethyl sulfoxide (DMSO), and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St Louis, MO, USA). A commercial cyclooxygenase (COX-2) inhibitory screening assay kit, Cayman test kit- 701230 (Cayman Chemical Company, Ann Arbor, MI, USA).

2.3. Total lipid extraction

The total lipid extraction of each microalgae culture was carried out according to the Folch method [40]. 25 mg of the dry biomass of each microalgae was mixed with 2.0 mL of CH_2Cl_2:MeOH (2:1, v/v) in a glass tube and homogenized by vortex for 2 min and incubated on a heating block at 30 ºC for 30 min. After incubation, the mixture was centrifuged (Selecta JP Mixtasel, Abrera, Barcelona, Spain) for 10 min at 2000 rpm and the organic phase was collected in a new glass tube. The biomass residue was extracted again four times. The combined organic phases were dried under a stream of nitrogen gas. To wash the lipid extract and induce phase separation, the initial extract was redissolved in 2 mL of CH_2Cl_2, 1 mL of MeOH and 0.75 mL of Milli-Q water, followed by a 2 min vortex and 10 min centrifugation at 2000 rpm. The lower organic phase was collected in a new pre-weighed glass tube. The aqueous phase was re-extracted three times with 2 mL of CH_2Cl_2, then vortexed for 1 min and centrifuged for 10 min at 2000 rpm. The combined organic phases were dried under a stream of nitrogen and weighted. The lipid extracts were then transferred to pre-weighted amber vials, dried again, weighed, and stored at −20 °C. The lipid content was estimated as a percentage of dry weight and the results were expressed as mean ± standard deviation of 5 replicates.

2.4. Quantification of phospholipid by phosphorous measurement

The amount of phospholipids (PL) in the lipid extracts, was determined according to Bartlett and Lewis [41]. Phosphate standards of 0.1 to 2 µg of phosphorous were prepared from a solution of monosodium phosphate (100 µg/mL, $NaH_2PO_4.2H_2O$). Dried aliquots of samples and standards were re-suspended in 125 µL of 70% perchloric acid. The samples were then heated to 180 °C in a heating block (Stuart, Staffordshire, UK), for 60 min, then cooled to room temperature. Subsequently, 825 µL of milli-Q water, 125 µL of 2.5% ammonium molybdate solution (2.5g of ammonium molybdate in 100 mL of Milli-Q water) and 125 µL of ascorbic acid 10% (10g of ascorbic acid in 100 mL of Milli-Q water) were added to the samples and standards, vortexed after each addition and incubated for 10 min at 100 °C in a water bath. After cooling, the absorbance of the samples and standards were measured at 797 nm in a ultraviolet–visible (UV-Vis) spectrophotometer (Multiskan GO, Thermo Scientific, Hudson, NH, USA). The percentage by weight of phospholipid (PL) was calculated by multiplying the quantity of phosphorus (µg) by 25. Two duplicates of two independent measurements were performed for each growth condition.

2.5. Glycolipid quantification by orcinol colorimetric method

To determine the glycolipid content of the lipid extracts, the orcinol assay was carried out according to CyberLipids [42]. The Glycolipid content was obtained by calculating the hexose content (% of glucose). The amount of sugar was read from a standard curve prepared by carrying out the reaction on known amounts of glucose (between 0 and 50 µg, from an aqueous solution containing 2.0 mg/mL of D-glucose). The absorbance of standards and samples was measured at 505 nm in a UV-Vis spectrophotometer (Multiskan GO, Thermo Scientific, Hudson, NH, USA). Two duplicates of two independent measurements were performed for each growth condition.

2.6. Cyclooxygenase 2 inhibition assay

500 µg of the lipid extracts were dissolved in 100% DMSO. The extracts were then tested using a cyclooxygenase 2 (COX-2) inhibitory screening assay kit (Cayman test kit-701080, Cayman Chemical Company, Ann Arbor, MI, USA), according to the manufacturer

protocol. The COX-2 inhibitor screening assay directly measures the amount of prostaglandin F2α generated from arachidonic acid (AA, 20:4 ω6) in the cyclooxygenase reaction. The prostanoid produced was quantified by spectrophotometry (412 nm, Multiskan GO 1.00.38, Thermo Scientific, Hudson, NH, USA) and processed with the software SkanIT version 3.2 (Thermo Scientific). The results were expressed as a percentage of inhibited COX-2.

2.7. Antioxidant activity by the α,α-diphenyl-β-picrylhydrazyl radical assay

The antioxidant scavenging activity against the α,α-diphenyl-β-picrylhydrazyl radical (DPPH$^\bullet$) was evaluated as described previously [32,43] with some modifications. Briefly, 150 µL of an ethanolic dilution of the extracts (25, 125, 250, 500 µg/mL) or 150 µL of the Trolox standard solution (5, 12.5, 25, 37.5 µmol/L in ethanol) were mixed with 150 µL of a DPPH$^\bullet$ working solution in ethanol (absorbance ~0.9, 517 nm). The mixture was incubated for 120 minutes and the absorbance was measured at 517 nm every 5 min (Multiskan GO 1.00.38, Thermo Scientific, Hudson, NH, USA). A control was prepared by replacing the DPPH$^\bullet$ solution with ethanol. The analyzes were carried out in triplicate. The antioxidant activity, expressed as a percentage of inhibition of the DPPH$^\bullet$ radical, was calculated using the equation 1:

$$\text{Inhibition\%} = \frac{\left(Abs_{DPPH\bullet} - \left(Abs_{Sample} - Abs_{Control}\right)\right)}{Abs_{DPPH\bullet}}\ x\ 100\ (1)$$

The activity was expressed in Trolox Equivalents, and calculated using equation 2, where the IC50 values are the concentration of the sample or of Trolox which induces the reduction the DPPH$^\bullet$ radical to 50%:

$$\text{TE (µmol/g)} = \frac{\text{IC50 Trolox (µmol/g)}}{\text{IC50 of samples (µg/mL)}}\ x\ 1000\ (2)$$

2.8. Antioxidant activity by the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay

The antioxidant scavenging activity against the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) was evaluated as previously described [43,44] with some modifications. Briefly, 150 µL of an ethanolic dilution of the extracts (25, 125, 250, 500 µg/mL) or 150 µL of the Trolox standard solution (5, 12.5, 25, 37.5 µmol/L in ethanol) were mixed in triplicate with 150 µL of an $ABTS^{\bullet+}$ working solution in ethanol (absorbance ~0.9, 734 nm). The mixture was incubated for 120 minutes and the absorbance was measured at 734 nm every 5 min (Multiskan GO 1.00.38, Thermo Scientific, Hudson, NH, USA). A control was prepared by replacing the $ABTS^{\bullet+}$ solution with ethanol. The antioxidant activity, expressed as a percentage of inhibition of the ABTS radical, was calculated using equation (1) (Abs_{DPPH} substituted by Abs_{ABTS}) and in Trolox equivalents using equation (2).

2.9. Fatty Acid Analysis by Gas Chromatography-Mass Spectrometry

The fatty acid methyl esters were prepared from total lipid extracts as previously reported [45]. Gas chromatography-mass spectrometry (GC–MS) analyses were performed on an Agilent 6890 N gas chromatograph interfaced with an Agilent 5973 mass spectrometer (Agilent, Santa Clara, CA, USA) with electron impact ionization (70 eV). A DB-FFAP capillary column (30m x 0.32 mm, 0.25 µm film thickness (J&W Scientific, Folsom, CA, USA)) was used. The following conditions were used: helium as carrier gas (constant flow 1.4 mL min^{-1}), inlet temperature 220 °C, detector temperature 280 °C, injection volume 2 µL (splitless). The oven temperature was programmed as follows: 80 °C for 3 min, 25 °C min^{-1} to 160 °C, 2 °C min^{-1} to 210 °C, 30 °C min^{-1} to 250 °C (held for 10 min). Five analytical replicates of each lipid extract from each C*hlorella vulgaris* were performed. The fatty acids were identified by comparing their retention times with those of commercial standards (Supelco 37 Component Fame Mix, Sigma-Aldrich), and by matching their mass spectral fragmentation patterns with the standards and corresponding data (Wiley 275 library and AOCS lipid library). The relative abundance (RA) of FAs was calculated by the percent area method without using correction factors [46,47]. The RA of fatty acids was calculated by integrating the area under the peak and dividing the results by the sum of all areas of the identified FAs (using equation 3). In equation 3 A_{FA} is the peak area of the fatty acid, A_T is the sum of the peak areas of all fatty acids. The results were expressed as mean ± standard deviation.

$$\%\mathrm{FA} = \frac{A_{FA}}{A_T}\, x\, 100 \qquad (3)$$

2.10. Hydrophilic interaction liquid chromatography-mass spectrometry

The lipid extracts were analysed by hydrophilic interaction liquid chromatography (HILIC) in a Ultimate 3000 Dionex (Thermo Fisher Scientific, Bremen, Germany) with an autosampler coupled to the Q-Exactive® hybrid quadrupole mass spectrometer (Thermo Fisher, Scientific, Bremen, Germany). The mobile phases A and B were composed of water/acetonitrile/methanol (25/50/25% and 0/60/40%, respectively) with 2.5 mM ammonium acetate. The following gradient was applied: 10% A (0-2 min), 10-90% A (2-15 min), 90% A (15-17 min), returning to the initial conditions in 3 min and held for more 10 min. A volume of 5 µL of each sample, containing 5µg of lipid extract, 4 µL of phospholipid standards (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine - 0.02 µg, N-palmitoyl-D-erythro-sphingosylphosphorylcholine (SM d18:1/17:0) - 0.02 µg, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (PE 14:0/14:0) - 0.02 µg, 1-nonadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC 19:0) - 0.02 µg, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylinositol (PI 16:0/16:0) - 0.08 µg, 1,2-dimyristoyl-*sn*-glycero-3-phospho-(10-rac-glycerol) (PG 14:0/14:0) - 0.012 µg, 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (PS 14:0/14:0)- 0.04 µg) and 91 µL of starting eluent (10% A), were loaded into the microbore column Ascentis® Si (10 cm × 1 mm, 3 µm, Sigma-Aldrich) at 35 °C and at a flow-rate of 50 µL min^{-1}.

The mass spectrometer operated in simultaneous positive (ESI 3.0 kV) and negative (ESI −2.7 kV) modes with the following configuration: resolution 70,000, automatic gain control (AGC) target 1×10^6, capillary temperature 250 °C, sheath gas flow rate was 15 U. In MS/MS experiments, the following configuration was used: top ten most abundant precursor ions, resolution 17,500, AGC target 1 x 10^5, dynamic exclusion 60 s, intensity threshold of 1 x 10^4, normalized collision energy™ of 25, 30 and 35 eV. Data acquisition was carried out using the Xcalibur data system (V3.3, Thermo Fisher Scientific, USA). Five independent biological replicas were performed and each replica was injected in duplicate. Identification of molecular species was based on the typical LC retention time, exact mass measurements, and manual interpretation of the LC-MS/MS spectra [22,48]. The semi-quantification was performed by integrating the area under the curve using the LC-MS chromatographic peak of each molecular ion. The peak areas were normalized by calculating the ratio concerning the content of GL or PL, evaluated using colourimetric tests, as described previously.

2.11. Statistical Analysis

The mean of five independent biological replicates ± standard deviation of the individual samples were compared. The integration of LC-MS chromatograms including baseline correction, peak deconvolution, deisotoping and alignment, and gap-filling was carried out using the MZmine 2.32 software package [49], followed by the normalization of the areas of integrated peaks by calculating the ratio against the content of glycolipids or phospholipids.

Multivariate and univariate analyses were performed using R version 3.5 [50] in Rstudio version 1.1.4 [51]. GC data and LC-MS data were glog transformed. Principal Component Analysis (PCA) was conducted for exploratory data analysis, with the R built-in function and ellipses were drawn using the R package ellipse [52], assuming a multivariate normal distribution and a level of 0.95. *Wilcoxon rank-sum test* was performed with the R built-in function. P-values were corrected for multiple testing using the BH Benjamini, Hochberg, and Yekutieli method (q values) [53]. Heatmaps and Hierarchical Cluster Analysis were created using the R package pheatmap [54] using "Euclidean" as the clustering distance, and "ward.D" as the clustering method. All graphics and boxplots were created using the R package ggplot2 [55].

3. Results

3.1. Comparative analysis of the lipid, phospholipid and glycolipid contents of lipid extracts from autotrophic and heterotrophic cultures of *Chlorella vulgaris*

The lipid content per dry weight (% DW) was 7.9 ± 0.7 for *Chlorella vulgaris* grown under heterotrophic conditions (C-Hetero) and 12.5 ± 0.9 for *Chlorella vulgaris* grown under autotrophic conditions (C-Auto) (**Table 2.1**). The content of phospholipids and glycolipids in the total lipid extracts was determined by colourimetric methods and the results are also summarized in **Table 2.1**. The results showed different amounts of phospholipids and glycolipids in the total lipid extract of C-Auto and C-Hetero. The maximum content of phospholipids was recorded in C-Hetero (18.4% versus 12.6 %) and the maximum content of glycolipids in C-Auto (47.5% versus 31.6%). The % of neutral lipids was estimated as the difference between the amount of the total lipids and the amount of phospholipids and glycolipids. The results showed that the higher neutral lipid content was in C-Hetero (50.0 %).

Table 2.1. The total amount of lipids, phospholipids, and glycolipids in the lipid extracts obtained from *Chlorella vulgaris* cultivated under heterotrophic and autotrophic conditions. Values represent the mean ± standard deviation of five independent experiments.

	Mean ± SD								
Growth conditions	Lipid total extract (mg)	Yield of lipid extraction (%)	GL (µg/mg)	% of GL	PL (µg/mg)	% of PL	% of polar lipids (sum of PL+GL)	% of neutral lipids + pigments	ratio of neutral/ polar lipids
C-Hetero	2.0 ±0.2	7.9 ±0.7	315.8 ±41.9	31.6 ±4.2	183.9 ±21.6	18.4 ±2.2	50.0 ±5.6	50.0 ±5.6	1.0 ±0.2
C-Auto	3.1 ±0.2	12.5 ±0.9	475.4 ±56.6	47.5 ±5.7	125.8 ±11.1	12.6 ±1.1	60.1 ±5.7	39.9 ±5.7	0.7 ±0.2
	q value								
C-Hetero vs C-Auto	0.016	0.016	0.013	0.013	0.013	0.013	0.034	0.034	0.034

Differences with *q* value < 0.05 were considered statistically significant. PL, phospholipids; GL, glycolipids, C-Auto, *Chlorella vulgaris* cultivated under autotrophic conditions, C-Hetero, Chlorella vulgaris cultivated under heterotrophic conditions, SD- standard deviation.

3.2. Comparative analysis of the fatty acids profile of *Chlorella vulgaris* cultivated under heterotrophic and autotrophic conditions

The fatty acid profile of the C-Auto and C-Hetero was mainly composed of fatty acids with a carbon side chain length of 16 and 18 (**Table 2.2** and **Supplementary Figure S2.1**). The fatty acid profile of C-Auto showed a higher amount of polyunsaturated fatty acids (PUFAs) than C-Hetero with an average of 57.5% versus 43.9%, respectively. Interestingly, C-Auto was mainly composed of *n*-3 PUFAs and C-Hetero was mainly composed of *n*-6 PUFAs. The first, second, and third most abundant fatty acids in C-Hetero were linoleic acid (C18:2*n*-6), palmitic acid (C16:0) and oleic acid (C18:1) which contributed with 27%, 24% and 19% of the total pool of FAs, respectively. The most abundant fatty acids in C-Auto were α-linolenic acid (C18:3*n*-3), palmitic acid (C16:0), linoleic acid (C18:2*n*-6) and 7,10,13-hexadecatrienoic acid (C16:3*n*-3), which contributed with 24%, 18%, 15% and 12% of the total pool of fatty acids, respectively. For the two total lipid extracts, monounsaturated fatty acids (MUFAs) were less abundant, 21% (C-Hetero) and 14% (C-Auto), than PUFAs and than saturated fatty acids (SFAs) (35% in C-Hetero and 29% in C-Auto). The higher abundance of the saturated fatty acids for heterotrophic conditions is mainly due to palmitic acid, (C-Hetero = 24% and C-Auto =18%).

Statistical analysis (Wilcoxon rank-sum test, FDR adjusted) was performed to identify significant changes in the fatty acids profile between culture conditions. The results showed significant changes in 9 of the 14 fatty acids identified, showing significant differences in lipid metabolism according to the growth conditions (**Table 2.2**). The most significant changes among C-Auto and C-Hetero were observed in the following fatty acids: C15:0, C16:0, C16:1 Δ^9, C16:3 (*n*-3), C17:1, C18:1, C18:2 (*n*-6), C18:3 (*n*-3) (**Figure 2.1A**); being observed statistically significant lower levels of C16:0, C18:1, C18:2 (*n*-6) in C-Auto.

Table 2.2. GC-MS analysis of the fatty acid composition of total lipid extracts of *Chlorella vulgaris* cultivated under heterotrophic and autotrophic conditions. Values represent the average ± standard deviation of five independent experiments.

	Mean ± SD		*q value*
Fatty acid	C-Hetero	C-Auto	C-Hetero vs C-Auto
C14:0	0.4 ± 0.0	0.5 ± 0.0	0.025
C15:0	0.2 ± 0.0	0.7 ± 0.0	0.014
C16:0	**24.2 ± 0.5**	**18.2 ± 0.7**	0.014
C16:1 Δ^7 (*n*-9)	1.7 ± 0.2	1.8 ± 0.1	
C16:1 Δ^9 (*n*-7)	0.3 ± 0.1	1.7 ± 0.1	0.014
C16:2 $\Delta^{7, 10}$ (*n*-6)	6.7 ± 0.6	6.3 ± 0.5	
C16:3 $\Delta^{7, 10, 13}$ (*n*-3)	3.6 ± 0.3	**12.5 ± 1.0**	0.014
C17:0	1.6 ± 0.2	1.5 ± 0.1	
C17:1	0.2 ± 0.1	1.1 ± 0.1	0.014
C18:0	8.0 ± 4.1	7.6 ± 2.9	
C18:1	**19.1 ± 2.1**	9.2 ± 1.0	0.014
C18:2 $\Delta^{9,12}$ (*n*-6)	**26.9 ± 1.6**	**15.1 ± 0.9**	0.014
C18:3 $\Delta^{9, 12, 15}$ (*n*-3)	6.8 ± 0.4	**23.6 ± 1.2**	0.014
C20:0	0.5 ± 0.1	0.3 ± 0.1	
Σ SFA	34.9 ± 4.5	28.8 ± 3.6	
Σ MUFA	21.2 ± 2.4	13.7 ± 1.1	0.013
Σ PUFA	43.9 ± 2.8	57.5 ± 3.5	0.013
Σ (*n*-3)	10.4 ± 0.6	36.1 ± 2.2	0.013
Σ (*n*-6)	33.5 ± 2.2	21.4 ± 1.3	0.013
n-6/*n*-3 ratio	3.2 ± 0.0	0.6 ± 0.0	0.013

Differences with *q* value < 0.05 were considered statistically significant. Σ SFA, the sum of saturated fatty acids; Σ MUFA, the sum of mono-unsaturated fatty acids; Σ PUFA, the sum of polyunsaturated fatty acids, C-Auto, *Chlorella vulgaris* cultivated under autotrophic conditions, C-Hetero, *Chlorella vulgaris* cultivated under heterotrophic conditions, SD- standard deviation. The most abundant FA are highlighted in bold font.

A statistical analysis was carried out to determine if the fatty acids signature could discriminate the growth conditions. Two-dimensional hierarchical cluster analysis (HCA) was applied to the GC-MS fatty acids profiles recorded for C-Auto and C-Hetero (**Figure 2.1B**). The primary split in the upper hierarchical dendrogram shows that the fatty acids data sets clustered independently in the two groups, *i.e.* C-Auto and C-Hetero. The clustering of individual fatty acids considering their similarity in the changes in relative abundance represented by the dendrogram on the left, also revealed that they were grouped into two main clusters. In the first group are represented the fatty acids which were more abundant in the two conditions. This was then divided into two groups, one containing C16:0, C18.1, and C18:2, which were more abundant in C-Hetero, and the other containing C16:3 and C18:3 higher in C-Auto. The second group, which contained the least abundant fatty acids in the two conditions, had, among others, C15:0, C16.1, and C17:1 which were less abundant in C-Hetero.

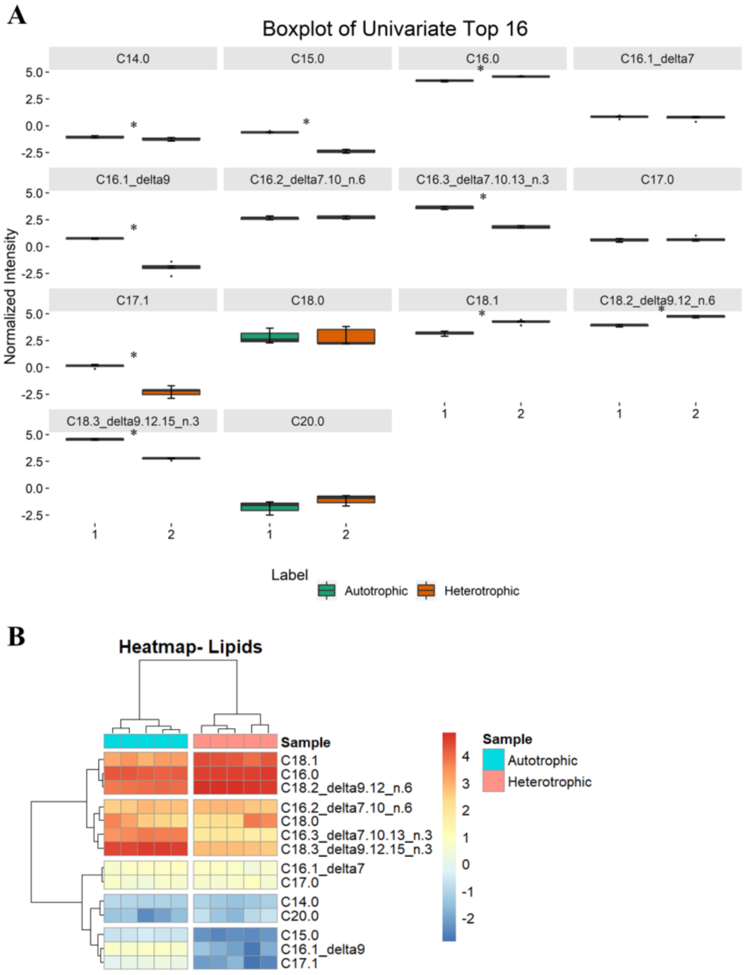


Figure 2.1. (**A**) Box plots of the GC-MS fatty acids (FA) profile recorded for *Chlorella vulgaris* cultivated under heterotrophic and autotrophic conditions, after Log normalization. * Statistically significant differences between growth conditions (Wilcoxon test, $q < 0.05$). (**B**) Two-dimensional hierarchical cluster analysis of GC-MS FA profiles. Relative abundance levels are indicated on the colour scale, with numbers indicating the fold difference from the mean. The clustering of the sample groups is represented by the dendrogram at the top, showing two main clusters, one for each group. The clustering of individual FA considering their similarity in relative abundance is represented by the dendrogram on the left.

3.3. Comparative analysis of the polar lipid composition of *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions

The polar lipidome of C-Auto and C-Hetero was analyzed by HILIC-ESI-MS and MS/MS. The lipidomic analysis showed that both lipid extracts were composed of the same lipid classes (resumed in **Table 2.3)**.

Table 2.3. Comparative analysis of polar lipid composition of total lipid extracts of *Chlorella vulgaris* cultivated under heterotrophic and autotrophic conditions, with the indication of the number of lipid species and lipid molecular species identified by the class of polar lipids. The number of lipid species is related to the molecular weight determined by MS and corresponds to the number of identified lipid molecular ions. The number of lipid molecular species is related to the interpretation of MS/MS data and attribution of the different composition of fatty acids to the lipid species.

Polar Lipid Classes	Number of lipid species identified in C-Auto	Number of lipid molecular species identified in C-Auto	Number of lipid species identified in C-Hetero	Number of lipid molecular species identified in C-Hetero
Phospholipids	**113**	**176**	**108**	**157**
PC	45	67	44	62
LPC	10	10	5	5
PE	31	55	31	47
LPE	7	7	4	4
PG	15	29	15	23
PI	5	8	9	16
Glycolipids	**58**	**86**	**57**	**84**
DGDG	21	32	20	34
DGMG	1	1	1	1
MGDG	18	35	18	31
MGMG	4	4	4	4
SQDG	14	14	14	14
Sphingolipids	**2**		**2**	**2**
Cer	1	1	1	1
PI_Cer	1	1	1	1
Total	**173**	**264**	**167**	**243**

C-Auto, *Chlorella vulgaris* cultivated under autotrophic conditions, C-Hetero, *Chlorella vulgaris* cultivated under heterotrophic conditions, PC, phosphatidylcholine, LPC, lysophosphatidylcholine, PE, phosphatidylethanolamine, LPE, lysophosphatidylethanolamine, PG, phosphatidylglycerol, PI, phosphatidylinositol, DGDG, digalactosyldiacylglycerol, DGMG, digalactosylmonoacylglycerol, MGDG, monogalactosyldiacylglycerol, MGMG, monogalactosylmonoacylglycerol, SQDG, sulfoquinovosyldiacylglycerol, Cer, ceramide, PI_Cer, inositolphosphoceramide. Numbers in bold are the sum of all species per lipid class.

In this work, we have identified six classes of phospholipids (phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) phosphatidylinositol (PI), lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE)), five classes of glycolipids (sulfoquinovosyldiacylglycerol (SQDG), digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), digalactosylmonoacylglycerol (DGMG) and monogalactosylmonoacylglycerol (MGMG)) and two classes of sphingolipids (ceramide (Cer) and inositolphosphoceramide (Pi_Cer)). All the lipid molecular species detected, the mean of areas of integrated peaks as well as their mass accurate mass measurement, are provided in the **Supplementary Table S2.1** to **Table S2.6**. **Supplementary Figure S2.2-Figure S2.6** show examples of representative LC-MS/MS spectra of each class of lipid, with the annotated identification of the main fragment ions that were used to identify the structure of polar lipids. The *sn*-position of fatty acids were assigned according to the most typical biosynthetic pathways. In phospholipids fatty acids with a lower acyl chain length are typically in the *sn*-1 position of the glycerol backbone and fatty acids with a longer acyl chain length are typically in the *sn*-2 position while in glycolipid the opposite usually is verified [56–59].

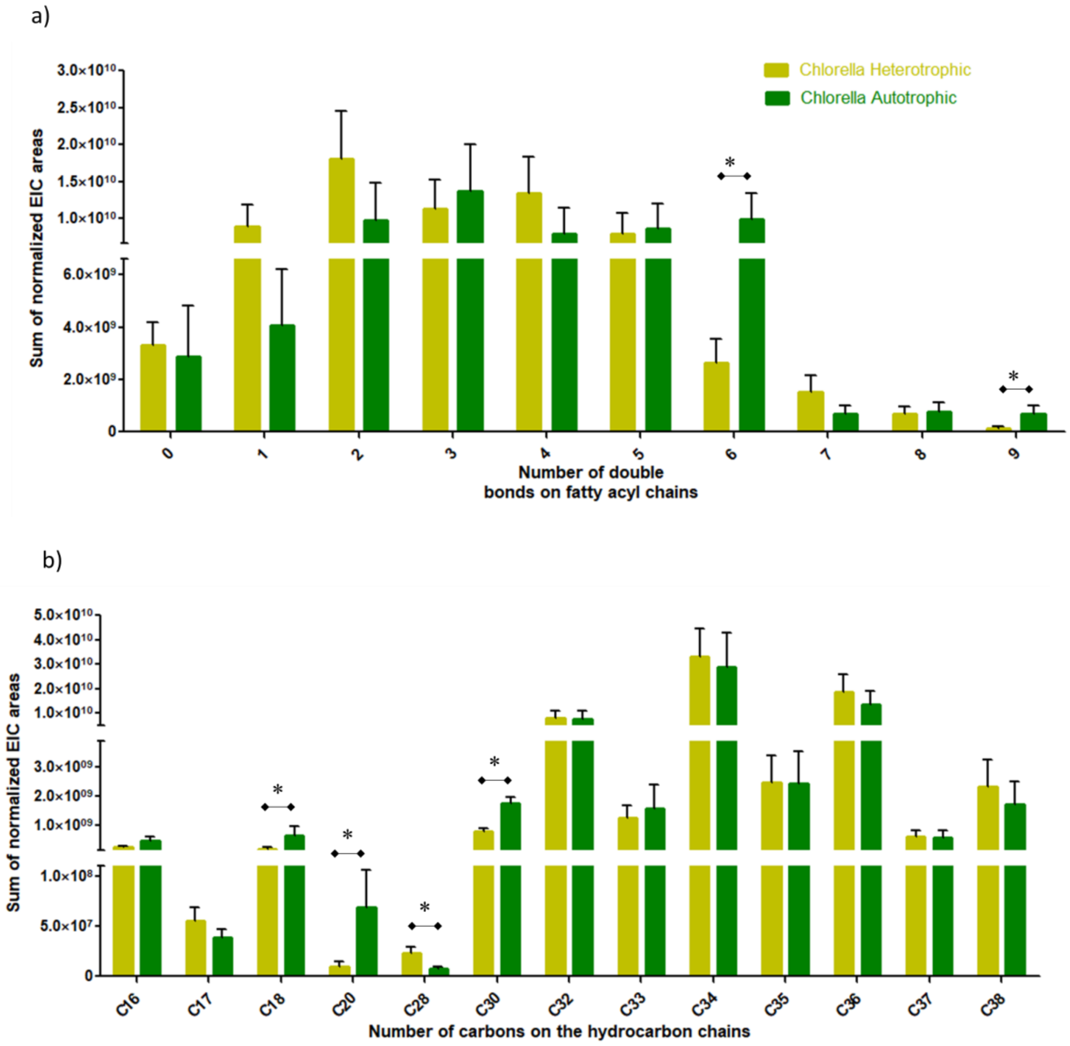
A total of 173 and 167 lipid species were detected for C-Auto and C-Hetero, respectively. Among them, 157 lipid species were the same under the two growth conditions (53 glycolipids, 102 phospholipids, and 2 sphingolipid species). A total of 44 and 45 lipid species of PC were detected for C-Hetero and C-Auto, respectively. For C-Auto samples, the most abundant phosphatidylcholine species were PC(36:6) and PC(36:5) and for C-Hetero were PC(36:4) and PC(34:2) (**Supplementary Figure S2.7a** and **Figure S2.8**). Regarding lysophosphatidylcholines, a total of 5 and 10 lipid species for C-Hetero and C-Auto, respectively, were identified. The lipid specie with higher abundance was LPC(18:2) under both conditions (**Supplementary Figure S2.7b**). Remarkably, the LPC(16:1), LPC(16:3), LPC(18:0), LPC(20:4) and LPC(20:5) species were only detected in lipid extracts of C-Auto. Overall, 7 lipid species of LPE and 31 of PE were detected in C-Auto and 4 lipid species of LPE and 31 of PE were detected in C-Hetero (**Supplementary Figure S2.7c**, **Figure S2.7d,** and **Supplementary Table S2.3**). LPE(16:1), LPE(18:3), and LPE(20:4) were detected only in C-Auto. LPE(18:1) was the lipid species with higher relative abundance in C-Auto while in C-Hetero was LPE(18:2). PE(34:2) was the most abundant species of PE for both conditions. PE(30:0) and PE(30:1) were detected only in C-Auto while PE(32:5) and PE(32:6) were detected only in C-Hetero.

We identified 15 common lipid species of PG in the lipid extracts of C*hlorella* from the two experimental groups (**Supplementary Table S2.4)**. Among these, the two most abundant lipid species under C-Hetero conditions were PG(34:1) and PG(34:2) and, under C-Auto conditions were PG(34:2) and PG(34:3) (**Supplementary Figure S2.7e** and **Supplementary Figure S2.9**). We identified 9 lipid species of PI, four of which were present only under C-Hetero conditions (PI(32.2), PI(36:2), PI(36:3) and PI(36:4)). The lipid species with higher abundance was PI(34:2) for both conditions (**Supplementary Figure S2.7f**).

In the lipid extracts of C. *vulgaris* from the two experimental groups, a total of 15 SQDG sulfoglycolipids species were detected. Of these, SQDG(36:6) was only found in C-Auto and SQDG(30:0) was only found in C-Hetero (**Supplementary Table S2.5**) and the most abundant species was SQDG(32:0) (**Supplementary Figure S2.10a**).

The neutral glycolipids identified corresponded to four classes of glycolipids, monogalactosyldiacylglycerol (MGDG), monogalactosylmonoacylglycerol (MGMG), digalactosylmonoacylglycerol (DGMG), and digalactosyldiacylglycerol (DGDG). A total of 19 MGDG lipid species were identified, but MGDG(32:6) was only identified in C-Auto and MGDG(34:7) in C-Hetero (**Supplementary Figure S2.10b**). A total of 5 MGMG lipid species were detected, of which MGMG(16:0) was only found in C-Hetero and MGMG(18:3) was detected only in C-Auto (**Supplementary Figure S2.10c**). A DGMG lipid species and a total of 22 DGDG lipid species were been identified, with DGDG(32:4) and DGDG(32:5) only detected in C-Auto and DGDG(36:2) only detected in C-Hetero (**Supplementary Figure S2.10d** and **Supplementary Table S2.6**). For C-Auto the most abundant species in each class were MGMG(18:3), MGDG(34:6), and MGDG(34:5), DGDG(34:6) and DGDG(34:5). For C-Hetero the most abundant species in each class were MGMG(16:3), MGDG(34:4), and MGDG(34:5), DGDG(34:2) and DGDG(34:1), (**Supplementary Figure S2.10** and **Figure S2.11**).

For the evaluation of the differences of the polar lipidome between the growth conditions, the lipids were grouped by the number of unsaturation (**Figure 2.2a**), the number of the total length of the carbon chain (**Figure 2.2b**) and the class of lipids (**Figure 2.2c**). The degrees of unsaturation observed for polar lipids of *C. vulgaris* species ranged from zero to nine double bonds. Among the polyunsaturated species, 1, 2. 4, and 7 unsaturated species were more abundant in C-Hetero and 3, 5, 6, and 9 in C-Auto, although only the differences in 6 and 9 unsaturations were statistically significant (**Figure 2.2a**). On the other hand, significant differences concerning the length of the carbon chain (C18, C20, C28, and C30) and the lipid class (DGDG, LPC, and PI) were also observed.



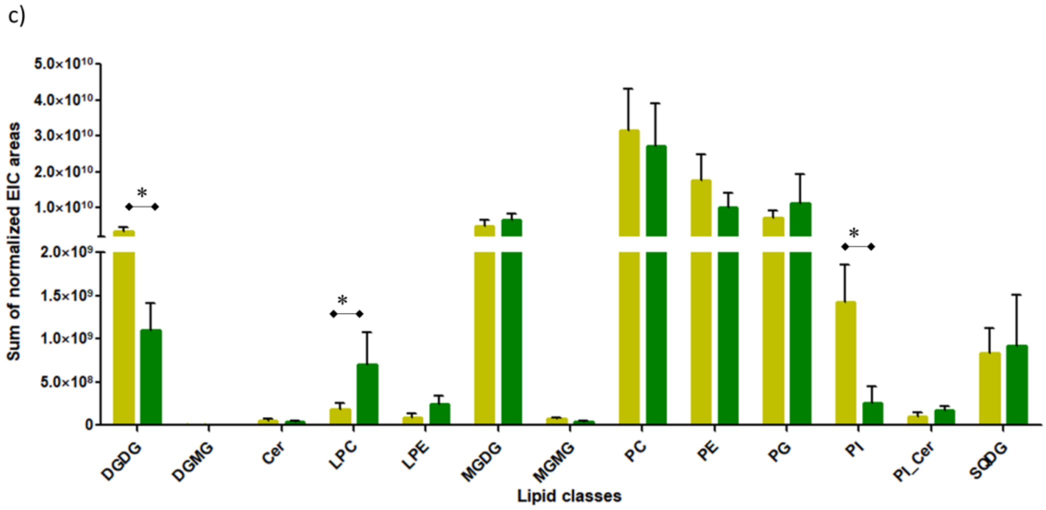


Figure 2.2. Lipid species of *Chlorella vulgaris* cultivated under heterotrophic conditions (in yellow) and *Chlorella vulgaris* cultivated under autotrophic conditions (in green) grouped by: **a**) degree of unsaturation; **b**) the carbon chain; and **c**) the class of lipids. * $q < 0.05$. PC, phosphatidylcholine, LPC, lysophosphatidylcholine, PE, phosphatidylethanolamine, LPE, lysophosphatidylethanolamine, PG, phosphatidylglycerol, PI, phosphatidylinositol, DGDG, digalactosyldiacylglycerol, DGMG, digalactosylmonoacylglycerol, MGDG, Monogalactosyldiacylglycerol, MGMG, monogalactosylmonoacylglycerol, SQDG, sulfoquinovosyldiacylglycerol, Cer, Ceramide, PI_Cer, inositolphosphoceramide.

The PCA analysis of the log-transformed levels of all the individual lipid species identified using the HILIC-MS/MS approach showed evident discrimination between the two experimental groups. The samples were clustered into two groups (C-Auto *vs* C-Hetero), with 98.2 % of the total variance in the data set (PC1 (87.8%), PC2 (10.4%)) (**Figure 2.3A**). As expected, the main contributors to discrimination were the lipid species detected in only one of the lipid extracts. The first sixteen species of PC1 were PE(30:0), PE(30:1), PE(32:6), MGDG(32:6), PI(32:2), PI(36:3), LPE(16:1), SQDG(36:6), MGDG(34:7), PI(36:2), PC(34:5-OH), PI(36:4), LPC(16:3), SQDG(30:0), LPC(20:4), DGDG(32:5) (**Figure 2.3B**).

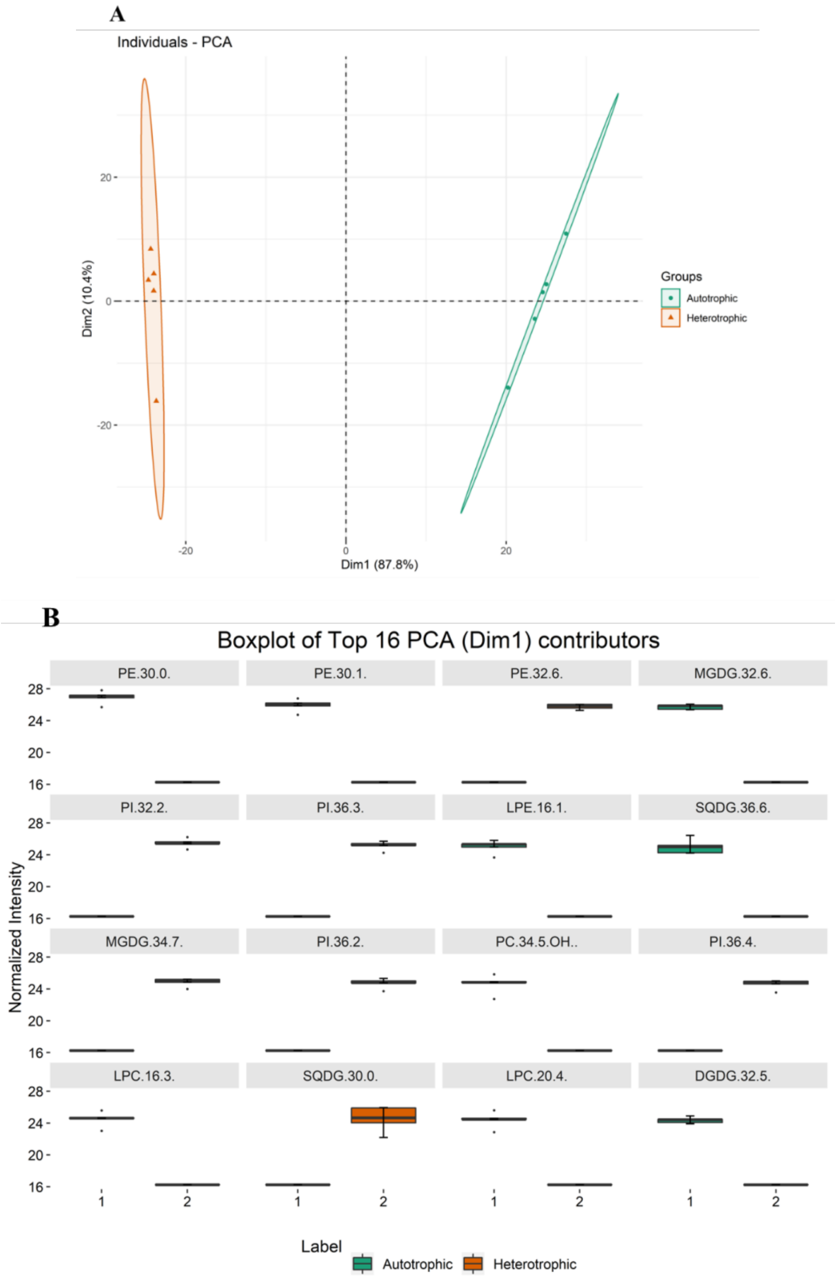


Figure 2.3. (**A**) Principal component analysis score plot of *Chlorella vulgaris* grown under autotrophic (green colour) and heterotrophic (orange colour) conditions, based on HILIC-MS/MS. (**B**) Box plots of the 16 main contributors for PC1. For all lipid species q<0.05. PC, phosphatidylcholine, LPC, lysophosphatidylcholine, PE, phosphatidylethanolamine, LPE, lysophosphatidylethanolamine, PI, phosphatidylinositol, DGDG, digalactosyldiacylglycerol, MGDG, Monogalactosyldiacylglycerol, SQDG, sulfoquinovosyldiacylglycerol.

As an additional approach, a PCA analysis using the lipid species only detected under the two culture conditions (a total of 157 lipid species) was performed. The results of the PCA analysis also revealed obvious discrimination between the two experimental groups, with 96.1 % of the total variance in the data set (PC1 66.2%, PC2 29.9%) (**Figure 2.4A**). The top sixteen contributors to PC1 discrimination were PG(34:4), PG(32:1), PG(34:3), PI(34:1), PC(38:9), PC(32:6), LPC(18:3), LPE(18:1) and glycolipids: MGDG(32:5), MGDG(36:6), MGDG(34:1), DGDG(35:1), DGDG(34:1), SQDG(32:3), SQDG(32:1), and SQDG(34:5) (**Figure 2.4B**).

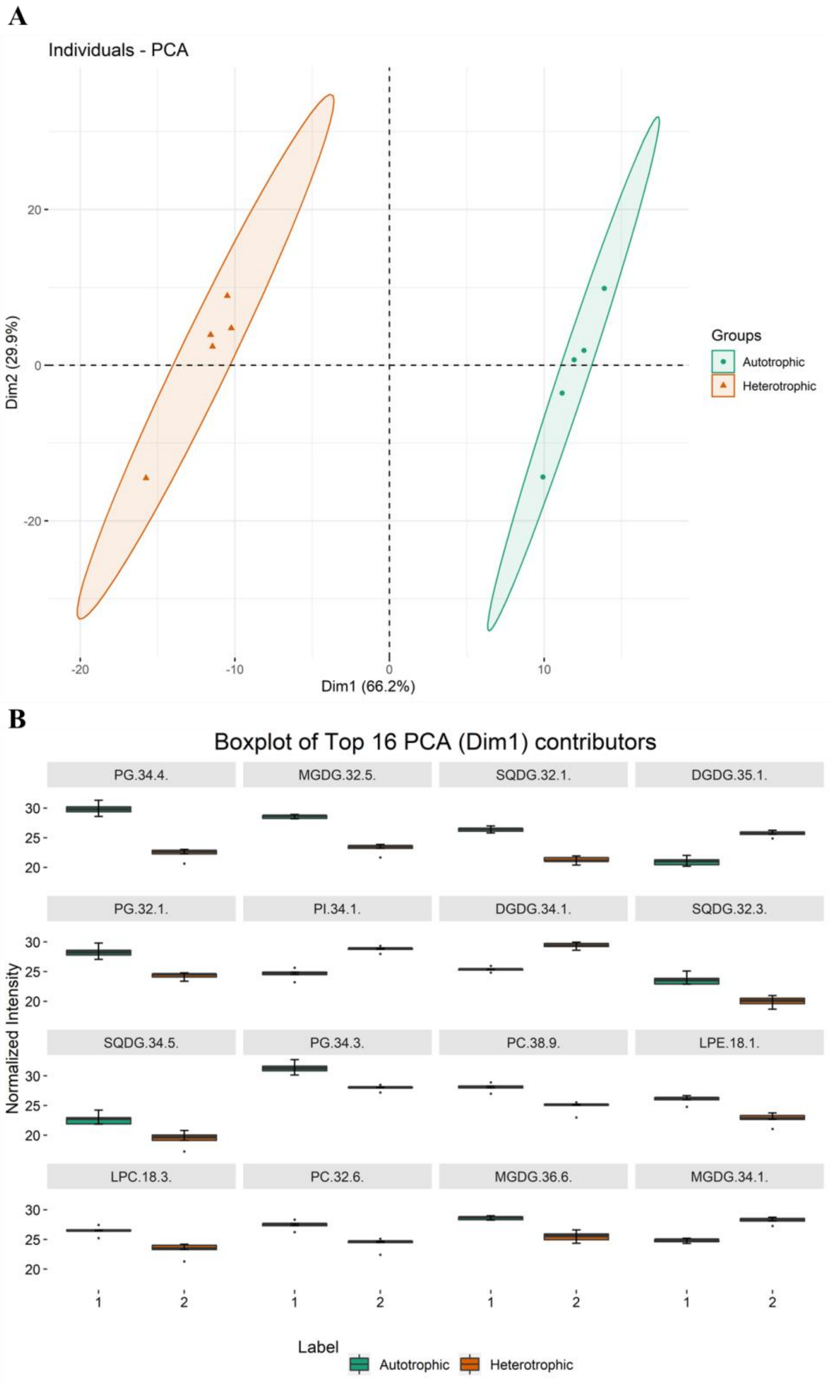


Figure 2.4. (**A**) Principal component analysis score plot of *Chlorella vulgaris* grown under autotrophic (green colour) and heterotrophic (orange colour) conditions, based on HILIC-MS/MS.

This dataset contains only the lipid species present in both conditions. (**B**) Box plots of the 16 main contributors for PC1. For all lipid species *q*<0.05. PC, phosphatidylcholine, LPC, lysophosphatidylcholine, LPE, lysophosphatidylethanolamine, PG, phosphatidylglycerol, PI, phosphatidylinositol, DGDG, digalactosyldiacylglycerol, MGDG, Monogalactosyldiacylglycerol, SQDG, sulfoquinovosyldiacylglycerol.

Finally, we constructed a dataset using the results of the *Wilcoxon rank-sum test* (**Supplementary Table S2.7**) to select the first 25 lower *q* values ($q < 0.05$) of the lipid species which were detected under the two conditions. This dataset was analyzed using HCA, and a dendrogram shown in **Figure 2.5**. The primary split in the upper hierarchical dendrogram shows that the polar lipid species data sets clustered independently in the two groups, namely C-Auto and C-Hetero. The clustering of the individual lipid species taking into account their similarity in the changes in the normalized amount represented by the dendrogram on the left, also revealed that they were grouped into two main clusters. The first group compiles the lipidic species upregulated in C-Hetero and downregulated in C-Auto and the second group the opposite. The main contributions of the lipid classes to the changes observed in the polar lipid profile between the experimental groups were the glycolipid classes (DGDG and MGDG) and the lysophospholipid classes (LPC and LPE) (**Figure 2.5**).

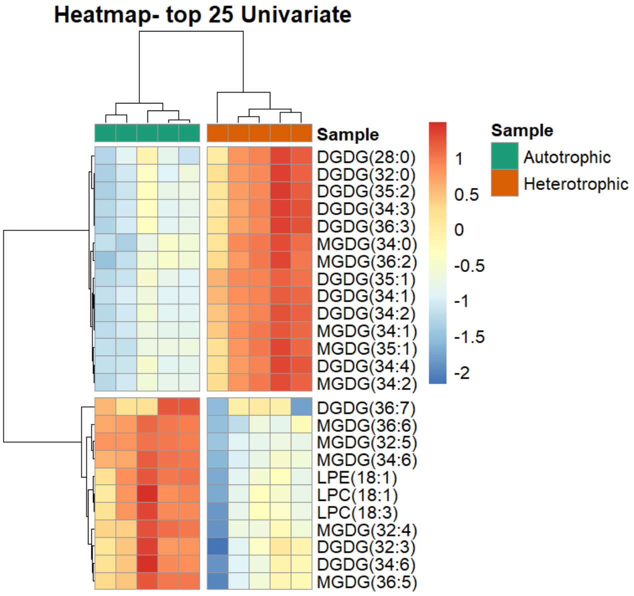


Figure 2.5. Two-dimensional hierarchical cluster heat map of *Chlorella vulgaris* grown under autotrophic (green) and heterotrophic (orange) conditions, based on HILIC-MS/MS. This dataset contains the lipid species that were significantly different between conditions (main 25 species) and

present in both conditions. The quantity levels are indicated on the colour scale, with numbers indicating the fold difference from the average. The clustering of sample groups is represented by the dendrogram at the top. The clustering of individual lipid species is represented by the dendrogram on the left. LPE, lysophosphatidylethanolamine, LPC, lysophosphatidylcholine, DGDG, digalactosyldiacylglycerol, MGDG, Monogalactosyldiacylglycerol.

3.4. Comparison between the bioactivities of the lipid extracts from *Chlorella vulgaris* cultivated under heterotrophic and autotrophic conditions

The antioxidant potential of the C-Auto and C-Hetero lipid extracts were evaluated using the α,α-diphenyl-β-picrylhydrazyl radical (DPPH$^{\bullet}$) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS$^{\bullet+}$) scavenging assays. No statistical differences were observed between the lipid extracts in the two antioxidant assays (**Table 2.4**).

Table 2.4. Antioxidant (ABTS and DPPH assays) and anti-inflammatory potential (COX-2 assay) using lipid extracts (12.5, 62.5, 125, 250 and 500 µg/mL) of *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions. No significant differences were found between conditions. The results were expressed as a percentage of inhibited ABTS, DPPH and COX-2.

	ABTS assay		**DPPH assay**		**COX-2 activity**	
Concentration of lipid extract (µg/ml)	C-Hetero (%)	C-Auto (%)	C-Hetero (%)	C-Auto (%)	C-Hetero (%)	C-Auto (%)
12.5	24.8 ± 2.00	27.5 ± 2.44	12.0 ± 2.08	15.5 ± 2.81	-	-
62.5	81.7 ± 3.23	76.2 ± 2.98	29.3 ± 1.04	29.4 ± 3.26	-	-
125	97.5 ± 0.44	92.9 ± 1.65	39.8 ± 0.59	42.1 ± 4.81	-	-
250	100.1 ± 0.11	99.7 ± 0.29	64.4 ± 1.47	58.5 ± 3.54	-	-
500	-	-	-	-	86.1 ± 0.47	86.5 ± 4.46

C-Auto, *Chlorella vulgaris* cultivated under autotrophic conditions, C-Hetero, *Chlorella vulgaris* cultivated under heterotrophic conditions, ABTS, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay, DPPH, α,α-diphenyl-β-picrylhydrazyl radical assay, COX-2, cyclooxygenase-2.

For the DPPH$^{\bullet}$ assay, the IC50 (Eq. (1)) of the C-Auto lipid extract was 197.2 ± 14.6 µg/mL and the TE (trolox equivalents) (Eq. (2)) was 110.2 ± 8.5 Trolox µmol/g lipid and for C-Hetero, the IC50 was 180.1 ± 5.7 µg/mL and TE was 120.1 ± 3.9 Trolox µmol/g lipid.

For the ABTS$^{\bullet+}$ assay, for C-Auto the IC50 was 51.1 ± 3.7 µg/mL and the TE was 313.1 ± 22.9 and for C-Hetero the IC50 was 51.5 ± 2.3 µg/mL and the TE was 310.0 ± 13.7 Trolox µmol/g lipid, respectively.

The anti-inflammatory potential of the lipid extracts of C. *vulgaris* grown under autotrophic and heterotrophic conditions was also determined. The two extracts showed anti-inflammatory activity. The 500 µg/mL of lipid extracts of C. *vulgaris* inhibited COX-2 activity to 86.1 ± 0.5 % (C-Hetero) and 86.5 ± 4.5 % (C-Auto) (**Table 2.4**). This difference in activity was not significantly different between the growth conditions.

4. Discussion

Chlorella vulgaris is a valuable source of phospholipids and glycolipids with omega-3 and 6 polyunsaturated fatty acids. *Chlorella* is known to have metabolic plasticity triggered by various biotic and abiotic factors, which leads to alterations in its biochemical composition [4]. Thus, by controlling the physical and chemical properties of the farming conditions, it is possible to modulate the production of the compounds of interest [4]. The lipidome of *C. vulgaris* grown under autotrophic conditions has already been reported [34]. However, the changes in its polar lipidome induced by culture under heterotrophic conditions have never been addressed. Heterotrophic growth has been studied because it overcomes the main limitations of autotrophic growth, such as light dependence, reduced production of products of interest, higher production costs and the difficulty of maintaining and controlling the growth of microalgae on a pilot scale, among others [16].

In this work, the lipid composition of the *Chlorella vulgaris* microalgae cultivated under heterotrophic and autotrophic conditions was studied. Cultivation of *Chlorella vulgaris* under autotrophic conditions resulted in a higher total lipid content than under C-Hetero conditions (13% *versus* 8%). However, the lipid yield obtained for C-Auto was lower than that previously reported, which was around 20% [7]. These results may be due to different cultivation conditions which may lead to a down-regulation of glycolysis and lipid biosynthesis, and up-regulation of gluconeogenesis [60]. Also, these results are similar to the total amount of lipids reported for *Chlorella saccharophila* and *Chlorella protothecoides*, which were higher under heterotrophic conditions [10,61,62]. The divergence of results between studies confirms that the accumulation of lipids in microalgae depends on the culture conditions and the microalgae species and that these changes are very variable. In addition, it is known that autotrophic cells are characterized by a high concentration of pigments, namely chlorophyll and carotenoid [10], which are liposoluble compounds. These compounds are present in total lipid extracts and can contribute to the total lipid content [10]. However, the heterotrophic culture condition leads to a lower pigment content, and the liposoluble compounds are mainly lipid molecular species [10].

A higher phospholipid content in C-Hetero (18.4% *vs* 12.6%) and higher GL content in C-Auto (47.5% *vs* 31.6%) were observed. The higher glycolipid content in C-Auto could be a consequence of increased photosynthetic activity in autotrophy. Glycolipids are the main components of chloroplast membranes and play crucial functions in photosynthesis [63]. On the other hand, the increased content of phospholipids under heterotrophic culture conditions revealed the best potential of this condition for cosmeceutical and cosmetic formulations. In these industrial sectors, extracts rich in phospholipids are very necessary, because of its properties as emulsifiers, thickening agents, and antioxidants. Additionally, the maximum content of neutral lipids was recorded in C-Hetero (50.0% *vs* 39.9%). These observations are consistent with previous work [14,15], which reported an increase in neutral lipids under this growth condition [14,15].

The GC–MS fatty acid analysis showed that C-Auto was characterized by a higher level of PUFAs (57.5% *vs* 43.9%), in particular in omega-3 fatty acids (C18:3*n*-3 and C16:3*n*-3). These results are consistent with previous studies [62]. Otherwise, C-Hetero was characterized as having a higher content of more saturated fatty acids (35% *vs* 29%), a lower amount of omega-3 PUFAS ($\sum n$-3 =10% *vs* 36%), and a higher quantity of omega-6 PUFAS ($\sum n$-6 =34 % vs 21%). The higher abundance of saturated fatty acids for heterotrophic conditions is mainly due to palmitic acid (C-Hetero = 24% and C-Auto =18%). These results are consistent with previous results obtained with *Chlorella homosphaera*, *Chlorella* sp. and *Chlorella minutissima* [12]. These authors reported that, for example for *Chlorella* sp., the palmitic acid content varied from 14.5% (without glucose) to 20.8% (with glucose). Also, growth under heterotrophic conditions favoured an increase in the cumulative levels of (poly)unsaturated species with 2 double bonds and downregulation of (poly)unsaturated species with 6 double bonds compared to C-Auto [12]. Although some studies reported the presence of the fatty acids C20:4 and C20:5 in *Chlorella Vulgaris* [64–66], we were unable to detect them. Nevertheless, we were able to dected lysophosphatidylcholines and lysophosphatidylethanolamines species with these fatty acids by LC-MS analysis of C-Auto.

Phspholipid and glycolipid species have become high-value lipids with nutritional and potential health benefits [22]. Thus, the complete characterization of the phospholipids and glycolipids species of the lipidome of the C-Auto and C-Hetero cultures was acquired using HILIC-ESI-MS analyses. Several experiments have shown that individual lipid species in the same class of polar lipids have nearly identical response factors [67]. In fact, studies have shown that, in MS, the ionization efficiency of lipid species is dependents mainly on their polar head group and that the structure of the fatty acyl chains only slightly affects their ionization [68,69]. The HILIC-ESI-MS and MS/MS analysis allowed us to identify a total of 173 and 167 lipid species for C-Auto and C-Hetero, respectively, belonging to 13 classes of lipids. Previous work has detected a total of 67 lipid species of polar lipids in lipid extracts of *Chlorella vulgaris* under autotrophic conditions [34]. Also, in the present study, we report for the first time the presence of two classes of glycolipids (1 DGMG and 4 MGMG) and sphingolipids (1 ceramide and 1 inositol phosphoceramide). However, did not detect the presence of lyso-PG species as previously reported (a total of 3 lipid species) [34].

Two complementary statistical analyses were carried out to assess the clustering of the polar lipidomes identified under the two culture conditions. The multivariate analysis performed using all the lipid species detected showed significant alterations in the polar lipid profile between growth conditions. The boxplots of the 16 most discriminating lipid species of C-Auto vs C-Hetero highlighted the lipid species which were only detected in one of the lipid extracts, which belonged to glycolipids (MGDG and SQDG) and phospholipids classes (PE, PC, PI, LPC, and LPE). PI, LPC, and LPE play an important role as signalling molecules [70]. A higher number of PI lipid species were identified in C-Hetero, and in a total of 9 PI species, 4 of them were not detected in autotrophic cultures and were found in the top 16, corresponding to (PI(32.2), PI(36:2), PI(36:3) and PI(36:4)). Additionally, except for PI(34:3), all of the PIs detected in the two lipid extracts were found in larger quantities in the C-Hetero samples. Previous studies have reported an increase in phosphoinositide activities in plants during stress and also an accumulation of PIs in plants during dehydration conditions [70]. Under heterotrophic conditions, supplementing the cultures with a carbon source could contribute to the dehydration of the cultures and consequently lead to an increase in the PIs species in the lipid extracts of C-Hetero.

A higher number of LPC and LPE was detected in the C-Auto lipid extracts. Interestingly, the common lysophospholipids detected in the two lipid extracts were also all increased in the C-Auto lipid extracts. According to the literature, lysophospholipids are increased in injured plants and plants subjected to temperature acclimatization, in response to frost, infection by a pathogen, or the application of elicitors [70]. In a recent study that compared the lipidome of *Saccharina latissima* at different locations, the authors also observed an increase in the relative amount of lysophospholipid species at the location known to have some of the highest tides [71]. The results of our work allow us to suggest that the exposure of C-Auto samples to radiation could have induced the synthesis of lysophospholipids.

The multivariate analysis was then carried out with only the lipid species common between the growth conditions, also made it possible to discriminate the profile of the polar lipidome between the two culture conditions, observed in PCA analysis. The HCA of the 25 most significant species ($q<0.05$, **Figure 2.5**) shows that the discrimination between the conditions occurs mainly due to the glycolipid classes DGDG, MDGD, and lyso species. C-Hetero had a higher abundance of low unsaturated species, while C-Auto had a high abundance of highly unsaturated and lyso species. The glycolipid classes MGDG and DGDG, as well as the SQDG species, are major components of plastid lipids and are intimately involved in the photosynthesis process. Takács *et al* (2014) found that in infected leaves, a lower amount of highly unsaturated fatty acids and the reduced abundance of MGDG correlated with a reduction in the ratio of the prolamellar body (PLB) to the prothylakoid membrane and a disturbed structure of this compartment [72]. As a result, the synthesis of pigments in the greening process has been inhibited [72]. Our data are consistent with these observations in which, in heterotrophy, *C. vulgaris* does not dependent on photosynthesis, thus the structure of the prothylakoid membrane is altered and the biosynthesis of chlorophyll is inhibited.

All the analytical methods (colorimetric, GC-MS and HILIC-MS) that we have used provide different but complementary information. In our study we have observed the same tendency among the different methods. It was found an increase of PUFA with omega-3 through GC-MS and higher abundance of highly unsaturated polar lipids through LC-MS in C-Auto, as well as the increase of glycolipids proportion in C-Auto and higher abundance of specific glycolipid MGDG species (colorimetric and LC-MS methods). We have also observed an increase of phospholipid proportion in C-Hetero through colorimetric methods, and a higher abundance of PC and PE species through HPLC-MS.

Polar lipids have been associated with bioactivity properties and health benefits, such as anti-inflammatory, antioxidant, anti-tumor, and antimicrobial agents [22]. The most abundant lipid species of the sulfoquinovosyldiacylglycerol class for the two conditions, SQDG(32:0), was reported to have anti-tumor activity [28] and a potential activity against Alzheimer disease [73]. Furthermore, the monogalactosylmonoacylglycerol MGMG(16:3) and MGMG(16:2), in greater relative abundance in C-Hetero, have demonstrated anti-inflammatory activity [74]. Also MGDG(36:6) in greater relative abundance in C-Auto and

MGDG(34:2) and DGDG(34:2) in greater relative abundance in C-Hetero, have already been described as having anti-tumour activity [30].

Nowadays, in the cosmetic and other industrial sectors, there is a growing demand to find microorganisms capable of producing antioxidant compounds in the long-term [75,76]. These compounds are currently being studied for its applications such as the prevention of food oxidation, ageing processes and diseases. As such, the screening of microalgae extracts as sources of antioxidant compounds has been widely explored [75–81]. Thus, the potential antioxidant properties of the two lipid extracts of C*hlorella vulgaris* was evaluated using the ABTS and DPPH radical scavenging assays. The results gathered in our work from the $DPPH^{\bullet}$ assays showed that the chloroform-methanol extracts had an IC50 of 0.197 ± 0.01 mg/mL for C-Auto and 0.180 ± 0.06 mg/mL for C-Hetero, evidencing antioxidant properties. Moreover, these antioxidant properties of the extracts were corroborated by the results of the $ABTS^{\bullet +}$ test, showing very similar IC50 values among the chloroform-methanol extracts (0.051 ± 0.004 mg/mL for C-Auto and 0.051 ± 0.002 mg/mL for C-Hetero). No significant difference was observed between the extracts. Some studies have already reported antioxidant activity for C*hlorella vulgaris* extracts [77,79,81]. Recently, it has been reported that using the $DPPH^{\bullet}$ assay, C*hlorella vulgaris* ethanolic extracts has an IC50 of 108.6 mg/mL [77]. Besides, the two extracts showed better antioxidant activity than the methanolic extracts of the microalgal biomass of *Chlorella sorokiniana*, *Phaeodactylum tricornutum*, *Tetraselmis chuii*, *Nannochloropsis granulata*, *Botryococcus braunii*, *Neochloris oleoabundans*, *Porphyridium aerugineum*, *Scenedesmus obliquus* and *Scenedesmus* sp. [82].

Algae extracts have been a source of potential anti-inflammatory compounds, including glycolipids [22]. Here, we also evaluated the anti-inflammatory potential of the two lipid extracts (C-Auto and C-Hetero). We observed an inhibition of cyclooxygenase-2 (COX-2) activity of 85% and 86 % with the lipid extracts of C-Auto and C-Hetero at a concentration of 500 µg/mL, respectively. In a recent study, COX-2 inhibition of 79 ± 7% was reported for lipid extracts of *Isochrysis galbana*, however, these values were obtained with samples ten times more concentrated (5000 µg/mL) [83].

Overall, the heterotrophic growth of the microalga *Chlorella vulgaris* is probably more suitable for the production of biodiesel, due to the high content of saturated fatty acyl chains in the lipid extracts obtained from this culture condition [84]. Also, the higher amount of oleic acid in C-Hetero compared to C-Auto will contribute to the oxidative stability of biodiesel [15]. Previous studies have shown that a higher growth rate of microalgae biomass, as well as lower production costs, were associated with heterotrophic growth conditions [16,85].

However, from a pharmaceutical and nutraceutical point of view, the C-Auto culture could be the best for obtaining value-added compounds, given the higher amount of omega-3 fatty acids, mainly esterified in polar lipids. Also, our results showed an *n*-6:*n*-3 ratio of 3.2 for C-Hetero and 0.6 for C-Auto. It has been reported that a balanced diet with the appropriate consumption of omega-6 and omega-3 fatty acids is important for the maintenance of health. Although the optimal ratio *n*-6:*n*-3 may vary depending on the authors, a lower ratio is more desirable for the prevention of chronic diseases promoting a healthy lifestyle [86]. A high intake of omega-6 fatty acids has been associated with the risk of overweight, obesity and leads to hyperactivity and the high intake of omega-3 fatty acids has been associated with a reduced risk of overweight [87].

5. Conclusions

This work provided new information on the adaptability of *Chlorella vulgaris* to autotrophic and heterotrophic growth conditions and characterized the polar lipids biochemical adaptation. The autotrophic conditions lead to the production of lipid extracts rich in glycolipids and with a higher content of omega-3 polyunsaturated fatty acids. Otherwise, heterotrophic conditions lead to an increase in the proportion of phospholipid and the percentage of saturated fatty acids and omega-6 polyunsaturated fatty acids. The two growth modes lead to the synthesis of lipid species which were not present in the other condition. Further, the two extracts had high anti-inflammatory and antioxidant properties.

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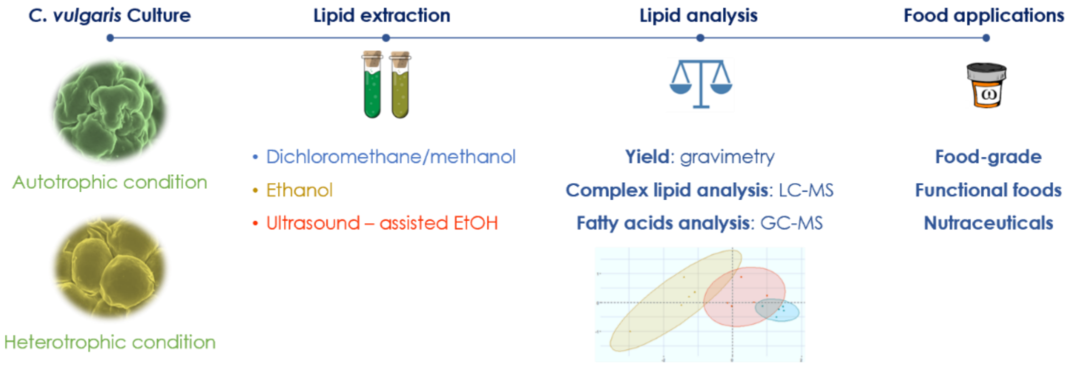
CHAPTER 3 - Food grade extraction of *Chlorella vulgaris* polar lipids: A comparative lipidomic study



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Food grade extraction of *Chlorella vulgaris* polar lipids: A comparative lipidomic study

Abstract: Glycolipids and phospholipids are the main reservoirs of omega polyunsaturated fatty acids in microalgae. Their extraction for the food industry requires food grade solvents, however, the use of these solvents is generally associated with low extraction yields. In this study, we evaluated the lipid extraction efficiency of food-grade ethanol, ultrasound-assisted ethanol (UAE) and dichloromethane/methanol (DCM) from *Chlorella vulgaris* cultivated under autotrophic and heterotrophic conditions. Yields of lipids, fatty acids (FA), and complex lipid profiles were determined by gravimetry, GC-MS, and LC-MS/MS, respectively. UAE and DCM showed the highest lipid yields with similar purity. The FA profiles were identical for all extracts. The polar lipidome of the DCM and UAE extracts was comparable, while the EtOH extracts were significantly different. These results demonstrated the effectiveness of UAE extraction to obtain high yields of polar lipids and omega-3 and -6-rich extracts from *C. vulgaris* that can be used for food applications.

Keywords: *Chlorella vulgaris*, Lipidomics, Lipid extraction method, Polar lipids, Neutral lipids, Pigments

1. Introduction

According to the FAO and Green Deal, there is a need to move towards a more sustainable and environmentally friendly global food system [1]. This transformation is an essential step in the fight against the scarcity of natural resources and the associated environmental impacts. This new sustainable food production should also offer a healthier diet and be more accessible to all, helping to reduce the risk of non-communicable diseases associated with unhealthy eating habits. The main risk factors for diseases linked to malnutrition are high consumption of red meat and ultra-processed foods, as well as, among others, low consumption of vegetables, fruits, and omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) [1]. Therefore, there is a growing concern in the food industry to develop foods enriched with omega-3 PUFAs or fortified with extracts rich in omega-3 for use as food supplements. To this end, microalgae produced in aquaculture have been recommended as alternative food products rich in healthy lipids [2]. The omega-3 PUFAs currently consumed are mainly extracted from fish oil, but the limits of fishing and the need for alternative sources for target populations, such as vegetarians and vegans, stimulate alternative sources of these nutrients [3].

Microalgae are rich in omega-3 and omega-6 lipids and a new trend either as a food or as a food ingredient. They can be produced on a large scale without competing with agriculture, as they can be grown on land not used for traditional agriculture and do not require significant land space. Microalgae can be produced in aquaculture under sustainable conditions, thus helping to reduce overexploitation of ocean resources. All of these benefits make microalgae a healthy and sustainable food and a promising omega-3 supplement. Among thousands of species of microalgae, *Chlorella vulgaris* is one of the most industrially cultivated [4], approved for novel foods, and studied worldwide [4]. *C. vulgaris* has a high lipid content (ranging from 5 to 68% dry weight [5]) and high amounts of omega-3 PUFAs, in particular, hexadecatrienoic acid (C16:3) and α-linolenic acid (ALA, C18:3), and omega-6 linoleic acid (C18:2, LA) [5]. Consumption of ALA has been reported to be associated with a lower risk of coronary heart disease [6] and type 2 diabetes mellitus [7]. In addition, lipids from microalgae are considered a unique source of omega-3 ALA for vegetarian and vegan diets [3]. LA is also important not only because it is an essential fatty acid, but also because it exhibits beneficial effects including anti-diabetic properties through its ability to inhibit protein tyrosine phosphatases associated with insulin resistance and activation of the AMPK and Akt pathways, as reported by Yoon *et al.* [8]. Previous work has shown that *C. vulgaris* can be enriched in ALA or LA depending on the growth conditions [9]. The heterotrophic culture pathway leads to an increase in omega-6 PUFAs, while omega-3 PUFAs are favoured under autotrophic growth conditions. Thus, manipulation of *C. vulgaris* cultivation conditions can enhance the extraction of omega-3 or -6 PUFAs to meet the needs of the food industry.

Lipid extracts from microalgae have been used for different applications, including food and nutraceuticals, but this extraction presents many challenges. For example, the most effective methods use organic solvents, which are not permitted in the food and feed industries due to the associated toxicity. This represents one of the biggest bottlenecks in the marketing of lipid extracts for food products.

Lipid extracts intended for food or pharmacological applications require food grade solvents. Ethanol (EtOH) is food grade, inexpensive, easy to handle, and environmentally friendly [10]. Thus, it has been used for *C. vulgaris* [11] and *Chlorella* sp. [12] lipid extraction, as well as other species of microalgae such as *Picochlorum* sp. [12] and *Nannochloropsis oceanica* [10]. However, the use of EtOH may result in lower lipid yield [10] or lower lipid purity [10,13]. To improve extraction yields, various cell disruption methods are used, such as pressurized fluid extraction, supercritical fluid extraction, microwaves, and ultrasound-assisted extraction [14]. Among them, ultrasound-assisted extraction, combined with EtOH, has provided encouraging results for *C. vulgaris* [11] and other microalgae such as *N. oceanica* [10]. However, most of the available data has focused on the efficiency of lipid extraction and the quality of the fatty acid (FA) profile of the extracted oil. On the other hand, the composition of polar lipids, phospholipids and glycolipids and triglycerides, as well as pigments remains to be explored. Omega-3 and omega-6 PUFAs are primarily found in polar lipids and are credited with promising bioactive properties and health effects, such as prevention of inflammation and cardiovascular disease [15] and cognitive brain development [16]. It also has other bioactive properties, including anti-inflammatory, antioxidant, and antineoplastic activities [17]. Consumption of polar lipids either in algae biomass or in extracts has been associated with health effects and these lipids are beginning to be used in food supplements and as food ingredients and functional food formulation [18].

In the present work, we sought to assess the efficiency of complex lipid extraction using ultrasound probe-assisted food-grade EtOH extraction (UAE) and EtOH from *C. vulgaris* grown under autotrophic and heterotrophic conditions. The results were compared with extracts obtained using dichloromethane/methanol (DCM).

2. Materials and methods

2.1. Microalgae strain and culture media

C. vulgaris 0002CA was provided by the Allmicroalgae industrial culture collection (Fábrica Cibra Pataias, 2445–287 Pataias, Portugal). *C. vulgaris* were grown under autotrophic (C-Auto) and heterotrophic (C-Hetero) conditions, as described previously [9]. Briefly, autotrophic cultures were grown in Guillard's F2 culture medium and cultured for 7 to 15 days in 5 L flask reactors under continuous light exposure. The 5 L flask reactors were used to inoculate a 1 m^3 L Flat Panel reactor, which was then used to inoculate a 10 m^3 photobioreactor and then sequentially scaled up to 70 m^3 photobioreactor. The photobioreactors were kept outdoors at constant pH.

Heterotrophic cultures were grown using a C: N ratio of 6.7:1 and glucose (Sapecquimica, Vila Nova de Gaia, Portugal). These cultures were obtained sequentially from 50 to 250 mL Erlenmeyer's to 5 L bench-top fermenter (New Brunswick BioFlo® CelliGen®115; Eppendorf AG, Hamburg, Germany), and later, to 200 L and 5000 L industrial fermenters. All the fermenters were kept under controlled temperature, dissolved oxygen and pH.

2.2. Reagents

HPLC grade dichloromethane (CH_2Cl_2), 96% absolute ethanol (CH_3CH_2OH), methanol (CH_3OH) and acetonitrile (CH_3CN) were purchased from Fisher Scientific Ltd. (Loughborough, UK). The water was of Milli-Q purity (Synergy1, Millipore Corporation, Billerica, MA). DPPH$^\bullet$ was purchased from Aldrich (Milwaukee, WI). All other reagents were purchased from major commercial sources. Phospholipid internal standards were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). The phospholipids used were: 1,2-dimyristoyl-*sn*-glycero-3-phospho-(10-rac-glycerol) (dMPG), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (dMPC), 1-nonadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylinositol (dPPI), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (dMPE), and N-heptadecanoyl-D-erythro-sphingosine (Cer(17:0/d18:1)).

2.3. Total lipid extraction

Lipid extraction was carried out using different solvent systems: ethanol (EtOH), EtOH extraction assisted by an ultrasound probe (UAE), and dichloromethane: methanol (2:1, v/v) (DCM), as described previously [10]. Briefly, 75 mg of spray-dried biomass was extracted with 2 mL of solvent (37.5 mg/mL) four times. For the UAE extraction, samples in EtOH were sonicated (Sonics VCX 130, Sonics & Materials INC., Newtown, CT., USA) using a microtip probe and output power of 130 W, output frequency 20 kHz, power density 3.56 W/cm^3, time 8 min, as previously reported [10]. The resulting supernatants were combined, filtered through Whatman filter paper No. 1., and dried under a stream of nitrogen and labelled as crude extracts. The extract yield (EY) was calculated using Eq. (1).

$$Extract\ yield\ \left(\%DW, \frac{w}{w}\right) = \frac{Weight\ of\ the\ extract\ (mg)}{Weight\ of\ biomass\ (mg)} x100 \text{ Eq. (1)}$$

The dried extracts were further subjected to Folch extraction (2 mL of dichloromethane, 1 mL of methanol and 0.75 mL of Milli-Q water), and the resulting organic phases were collected. The aqueous phase was re-extracted twice with 2 mL of dichloromethane. The combined organic phases were dried under a stream of nitrogen and labelled as lipid extracts. The lipid yield (LY) and the lipid content in the extract (LCE) were calculated using Eq. (2) and Eq. (3), respectively.

$$Lipid\ yield\ \left(\%DW, \frac{w}{w}\right) = \frac{Weight\ of\ the\ lipid\ extract\ (mg)}{Weight\ of\ biomass\ (mg)} x100 \text{ Eq. (2)}$$

$$Lipid\ content\ in\ the\ extract\ \left(\%DW, \frac{w}{w}\right) = \frac{Weight\ of\ the\ lipid\ extract\ (mg)}{Weight\ of\ extract\ (mg)} x100 \text{ Eq.(3)}$$

All contents (EY, LY and LCE) were further expressed as a percentage of the control, considering that the mean EY of DCM was 100 %. Five technical replicates were performed for each C-Auto and C-Hetero sample and each evaluated solvent system.

2.4. Scanning electron microscopy (SEM)

The microstructures of the microalgal biomass were analyzed by scanning electron microscopy (SEM) before and after extractions with different solvent systems (DCM, EtOH, UAE). The raw and extracted biomass powder samples (the latter left to dry for 72 h at room temperature in the fume hood) were dropped directly onto a double-sided carbon tape. The excess material was removed, and a thin film of conductive carbon was deposited on the powders using a carbon rod coater (Emitech K950X). SEM analyzes were performed on a SU-70 Hitachi microscope operated at 8kV.

2.5. Antioxidant activity

The antioxidant scavenging activity against the 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH$^\bullet$) was evaluated using a method previously described [9]. Briefly, 150 µL of an ethanolic dilution of the lipid extracts (25, 125, 250, 500 µg/mL) were mixed with 150 µL of DPPH$^\bullet$ working solution in EtOH (absorbance ~0.9). The control lipid extracts were tested by replacing 150 µL of DPPH$^\bullet$ diluted solution with 150 µL of EtOH. Absorbance was measured at 517 nm using a UV-vis spectrophotometer (Multiskan GO 1.00.38, Thermo Scientific, Hudson, NH, USA). All measurements were performed in triplicate. The antioxidant activity, expressed as a percentage inhibition of the DPPH$^\bullet$ radical, was calculated using Eq. (4):

$$Inhibition\ \% = \frac{(AbsDPPH-(AbsSample-AbsControl))}{AbsDPPH} x100 \text{ Eq. (4)}$$

The results were further expressed as a percentage of control, where the mean value of the per cent inhibition of DPPH obtained with the highest concentration of DCM extracts (250 µg/mL) was considered as 100 % and the other values were calculated accordingly.

2.6. Analysis of fatty acids by gas chromatography-mass spectrometry (GC–MS)

Methyl esters of fatty acids (FAMEs) were prepared from 30 µg of lipid extracts as previously described [9]. The dried extracts were dissolved with 1 mL of an internal standard of methyl nonadecanoate (1.0 µg mL^{-1}) (Sigma, St. Louis, MO, USA). Gas chromatography-mass spectrometry (GC–MS) analyses were performed on an Agilent Technologies 8860 GC System (Santa Clara, CA, USA) interfaced with an Agilent 5977B Mass Selective Detector (Agilent, Santa Clara, CA, USA) with electron impact ionization (70 eV) and scanning the range *m/z* 50–550 in a 1 s cycle in a full scan acquisition mode. A DB-FFAP capillary column (30 m x 0.32 mm, 0.25 µm; J&W Scientific, Folsom, CA, USA) was used. The following conditions were used: helium as carrier gas (constant flow rate 1.4 mL min^{-1}), inlet temperature 220 °C, detector temperature 230 °C. The oven temperature was programmed as follows: 58 °C for 2 min, 25 °C min^{-1} to 160 °C, 2 °C min^{-1} to 210 °C, 30 °C min^{-1} to 225 °C (held for 10 minutes). The data acquisition software used was GCMS5977B/Enhanced MassHunter. A qualitative data analysis software (Agilent MassHunter Qualitative Analysis 10.0) was used to identify FAs. The identification of FAs was performed by comparing retention times and mass spectra (MS) with those of commercial FAME standards (Supelco 37 Component FAME Mix, ref. 47885-U, Sigma-Aldrich, Darmstadt, Germany) and confirmed by comparison of the MS spectrum with the NIST library chemical database and by the literature. The relative abundance (RA, in percentage) of FAs was calculated as described previously [9]. Briefly, the RA of the FAs was calculated by integrating the area under the peak and divided by the sum of all the areas of the identified FAs.

2.7. Hydrophilic interaction liquid chromatography-mass spectrometry (HILIC–ESI–MS)

The lipid extracts were analyzed by hydrophilic interaction liquid chromatography (HILIC) in a Dionex Ultimate 3000 (Thermo Fisher Scientific, Bremen, Germany) using an Ascentis® Express column (10 cm x 2.1 mm, 2.7 µm; Sigma-Aldrich) coupled to the Q-Exactive® hybrid quadrupole Orbitrap mass spectrometer (Thermo Fisher, Scientific, Bremen, Germany). Mobile phase A consisted of H_2O:CH_3CN:MeOH (1:2:1 v/v/v), with 5 mM ammonium acetate, and mobile phase B was H_2O:CH_3CN:MeOH (0:3:2 v/v/v) with 5 mM ammonium acetate. The following gradient was applied: 5% A (0-2 min), followed by a linear increase to 48 % of mobile phase A in 8 min, and a further linear increase to 65% A in 5 min, followed by a 2-min hold period and return to initial conditions in 3 min for 10-min.

To perform HILIC–MS analyses, a volume of 10 µL of each sample (with 40 µg of lipid extract), 82 µL of eluent (95% of mobile phase B and 5% of mobile phase A), and 8 µL of phospholipid standards mixture (dMPC - 0.02 µg, dMPE - 0.02 µg, LPC - 0.02 µg, dPPI - 0.04 µg, dMPG - 0.012 µg, Cer(17:0/d18:1) - 0.02 µg), were mixed and a 5 µL of this mixture was injected into the Ascentis® Express microbore column at 35 °C and a flow-rate of 200 µL min^{-1}. The mass spectrometer was operated simultaneously in positive (ESI 3.0 kV) and negative (ESI −2.7 kV) modes as previously reported [9], except the sheath gas flow rate, which was 20 U. The MS/MS acquisitions were carried out as described in [9].

For lipidomic analysis, identification of (molecular) lipid species was based on the *m/z* ratio of the ions observed in LC-MS spectra, the mass accuracy (≤ 5 ppm), LC retention time, and manual interpretation of the LC-MS/MS spectra based on previous publications [9]. Ion peak integration was performed in MZmine software package version 2.53 and included baseline correction, peak deconvolution, deisotoping and alignment, and gap-filling as previously described [9]. Peaks of raw intensity lower than 1×10^4 were excluded. Semi-quantitation of lipid species was performed by dividing the integrated peak areas of each molecular ion by the peak area of the selected internal standards.

2.8. Statistical analysis

Multivariate and univariate analyses were performed using R version 4.0.2 [19]. A one-way ANOVA followed by the Tukey multiple comparison tests were performed to compare lipid contents and antioxidant activities. Whenever the normality assumptions were not verified, the Kruskal-Wallis test was employed. The Kruskal-Wallis test followed by Dunn's post-hoc comparisons were performed to compare the profile of the polar lipids, fatty acids, pigments, and triglycerides. Principal component analysis (PCA) and ellipses (level of 0.85) were performed for exploratory data analysis and were created using the R libraries FactoMineR [20]. The Kruskal-Wallis test followed by Dunn's post-hoc comparisons were performed with the R built-in function. The p-values were corrected for multiple testing using the BH Benjamin, Hochberg, and Yekutieli method (*q* values). A *q*-value < 0.05 was considered an indicator of statistical significance. Heatmaps and hierarchical clusters were created using the R package "pheatmap" and using "Euclidean" as clustering distance, and "ward.D" as the clustering method [21]. The heatmaps were constructed based on the 40 (polar lipids) or 18 (neutral lipids and pigments) lipid species with the lowest *p*-values in the Kruskal-Wallis test.

3. Results and discussion

3.1. The efficiency of lipid extraction from *Chlorella vulgaris* using different solvent systems.

Crude lipid extracts were obtained by extracting the biomass of *C. vulgaris* with EtOH, UAE, and DCM. Various parameters were calculated to determine the efficiency of the solvent systems, namely the extract yield (EY), the lipid yield (LY) and the lipid content of the extract (LCE) [10]. Extraction using DCM was used as a control experiment. Traditionally, the methods used to extract lipids from algae use Bligh Dyer or Folch with chloroform: methanol. In this work, we used dichloromethane instead of chloroform because dichloromethane is considered a safe alternative to chloroform giving a similar extraction yield [10] and some authors have reported better extraction efficiency using Folch´s method compared to Bligh and Dyer. Ethanol is a food grade and inexpensive solvent, but for some compounds like lipids, its extraction efficiency is limited. However, ethanol extraction assisted with ultrasounds has emerged as a green and environmentally friendly alternative extraction method. UAE has the advantage of being inexpensive, efficient, increasing the extraction yield without affecting the quality of the compounds.

The EY (mg of crude extract / 100 mg of biomass) and LY (mg of lipids extracted / 100 mg of biomass) obtained for UAE were higher in the case of C-Hetero compared to DCM, but lower in C-Auto (**Table 3.1**). LCE (mg of lipid extract / 100 mg of crude extract) was used to assess the recovery and purity of the lipid extracts. No statistical difference was detected between the lipid content of the extract when using the DCM and UAE methods (LCE> 80%), but these were statistically different from EtOH. This demonstrated the efficiency of UAE extraction to recover lipids from dry biomass of C-Auto and C-Hetero samples and to obtain pure lipid extracts.

The parameters evaluated showed that the association of an ultrasound probe with the EtOH extraction led to a significant increase in the efficiency of extraction of lipids from the biomass compared to EtOH and made it possible to obtain lipid extracts yields similar to the yields of conventional methods (DCM). These results are consistent with published studies that have used ultrasound as a useful tool to improve the lipid yields of *C. vulgaris* [11] and other species of microalgae [10,22]. The lowest LCE values (41.9 % for C-Hetero and 51.8 % for C-Auto) obtained with EtOH extraction are also consistent with the literature for other species of microalgae [10,13].

Table 3.1. *Chlorella vulgaris* extract yield (EY), lipid yield (LY) and lipid content of the extract (LCE) cultivated under heterotrophic (C-Hetero) and autotrophic (C-Auto) conditions, obtained using different solvent systems (DCM, EtOH and UAE). EY DCM was used as a control. The values were expressed as a percentage of the control and represent the mean ± standard deviation of five independent experiments.

		Mean ± STD			***q* values**		
		DCM	UAE	EtOH	DCM vs. UAE	DCM vs. EtOH	EtOH vs. UAE
EY (%)	C-Hetero	100.0 ± 6.9	132.0 ± 12.3	87.3 ± 17.3	*	ns	**
	C-Auto	100.0 ± 5.3	52.1 ± 2.8	20.8 ± 2.6	***	***	***
LY (%)	C-Hetero	92.7 ± 5.9	110.2 ± 9.2	35.8 ± 4.0	*	***	***
	C-Auto	90.6 ± 5.4	47.2 ± 4.8	10.6 ± 1.9	***	***	***
LCE (%)	C-Hetero	92.8 ± 1.2	83.6 ± 2.5	41.9 ± 5.7	ns	***	***
	C-Auto	90.8 ± 6.4	90.5 ± 7.1	51.8 ± 10.7	ns	**	**

DCM, dichloromethane: methanol (2:1, v/v); EtOH, ethanol: UAE, ethanol extraction assisted by an ultrasound probe; EY, extract yield; LC, lipid yield; LCE, lipid content of the extract; C-Hetero, C. vulgaris cultivated under heterotrophic conditions; C-Auto, C. vulgaris cultivated under autotrophic conditions. ns, not significant; q > 0.05; *q < 0.05; **q < 0.01; ***q < 0.001.

3.2. The effects of different extraction procedures on the cellular integrity of *Chlorella vulgaris* using scanning electron microscopy.

Cell integrity and microstructural changes of *C. vulgaris* before and after DCM, EtOH, and UAE extractions were studied by scanning electron microscopy (SEM) (**Supplementary Figure S3.1** and **Figure S3.2**). C-Auto cells did not have a well-defined shape. DCM extraction did not significantly affect the surface and shape of the cells but varied their surface roughness. EtOH and UAE extractions generally allowed cells to retain their original shape but increased their surface roughness due to surface attack [23,24]. C-Hetero cells exhibited an initial round shape, which persisted after EtOH and UAE extractions. UAE extraction contributed to the increase in surface roughness. DCM extractions lead to a specific loss of the round shape of cells that appeared to become interconnected.

Major structural cell surface changes were observed in cells extracted with UAE, with disruption signals, both in C-Auto and C-Hetero samples (**Supplementary Figure S3.1 C1-C2**). This result is in agreement with previous studies which reported the disruption of *C. vulgaris* [24] and *Chlorella* sp. [23] cells subjected to ultrasound-assisted extraction associated with cavitation and shock wave cell disruption. In the present work, the disruption of the *C. vulgaris* cell wall when subjected to UAE justifies the higher lipid contents observed once it promotes solvent penetration and lipid diffusion.

For efficient lipid extraction from microalgae, solvents must penetrate the cell membrane [25]. A mixture of solvents composed of an apolar solvent and a polar solvent, for example, dichloromethane and methanol, can cross the cell membrane more easily, having greater accessibility and efficiency in dissolving the membrane and intracellular lipids compared to a polar solvent (*e.g.* ethanol). In the case of UAE the cavitation phenomena increase the implosion of bubbles capable of causing cellular disruption (reviewed in [26], which promotes penetration of the solvent and increases the diffusion of lipids in ethanol. Interestingly, the extraction efficiency is different between C-Auto and C-Hetero both with ethanol and UAE extraction. This may be due to the different compositional properties and shapes of cell membranes, but more work is needed to clarify this issue. SEM images showed that the C-Auto and C-Hetero cells had different shapes, likely reflecting a different membrane composition and structure. This different composition can directly affect the efficiency of solvent extraction as well as the ability of ultrasound to promote solvent penetration.

3.3. Fatty acid profile of lipid extracts of *Chlorella vulgaris.*

The fatty acid (FA) profiles of the DCM, UAE, and EtOH extracts were determined by GC-MS. Eight FAs were identified, as previously reported [9]. Statistical analysis (Kruskal-Wallis test, FDR adjusted) was performed to assess the differences between the FA profiles. No statistical difference was observed in the FA profile between the DCM, UAE and EtOH extracts of C-Auto. In the case of C-Hetero, significant differences were observed only for the less abundant fatty acids (C16:0, C16:1*n*-9, C16:2*n*-6, C18:1*n*-9). Next, Dunn's multiple-comparison test (FDR adjusted) was performed to determine which pairs of solvent systems were significantly different. The results showed differences in the relative amount of FAs between DCM - EtOH and EtOH - UAE. No difference was observed between DCM and UAE (**Figure 3.1**). The results collected in our study were consistent with previously published data, which also reported small differences in the relative abundance of FAs from *C. vulgaris* lipid extracts obtained using chlorinated solvents and EtOH assisted by ultrasound [11]. However, other studies using different extraction methodologies have shown that these could significantly influence the FA relative abundances [27].

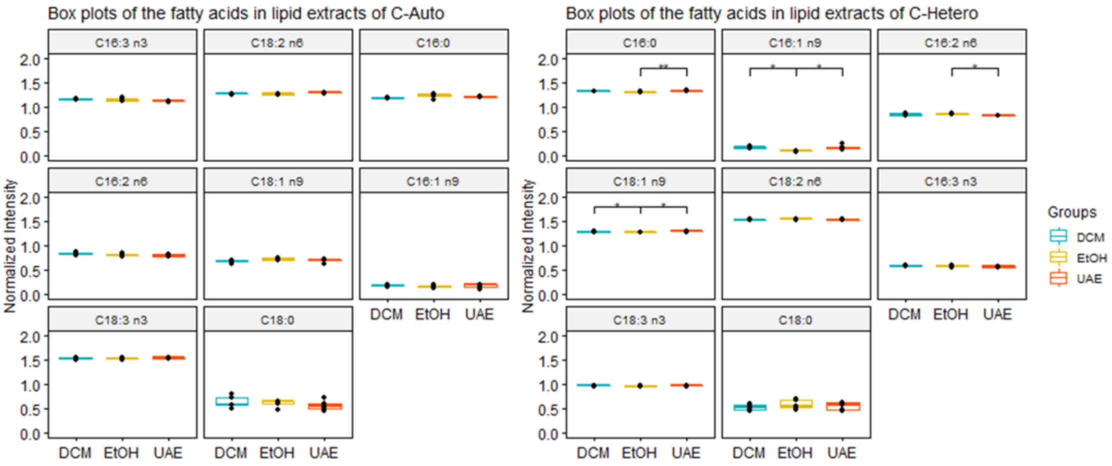

Figure 3.1. Box plots of the fatty acids identified in the DCM, EtOH, and UAE extracts of C-Auto (left panel) and C-Hetero (right panel). Abbreviations: C-Auto, *C. vulgaris* cultivated under autotrophic conditions; C-Hetero, *C. vulgaris* cultivated under heterotrophic conditions; DCM, dichloromethane: methanol (2:1, v/v); EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe. * $q < 0.05$, ** $q < 0.01$.

3.4. Profiling of polar lipids, triglycerides, and pigments in extracts of *Chlorella vulgaris*.

The lipid profiles of the lipid extracts of C-Auto and C-Hetero obtained using DCM, EtOH and UAE were analyzed and semi-quantified by HILIC-ESI-MS and MS/MS. This approach made it possible to identify and semi-quantify polar lipids, including phospholipids (PLs) and glycolipids (GLs), but also neutral lipids (triglycerides, TGs) and pigments. Using lipidomic approaches, we identified 172 and 167 species of polar lipids from all extracts of C-Auto and C-Hetero, respectively, from thirteen classes of polar lipids, as previously reported [9]: sulfoquinovosyldiacylglycerol (SQDG), digalactosyldiacylglycerol (DGDG), digalactosylmonoacylglycerol (DGMG), monogalactosyldiacylglycerol (MGDG), monogalactosylmonoacylglycerol (MGMG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), lysophosphatidylcholine (LPC) lysophosphatidylethanolamine (LPE), ceramide (Cer) and inositolphosphoceramide (PI_Cer). A complete list of polar lipid species detected and their accurate mass measurements are provided in **Supplementary Table S3.1** and **Table S3.2**. A total of 36 species of triglycerides (TG), 6 chlorophyll pigments, and 3 carotenoids were identified and quantified in all extracts. The carotenoids detected were: antheraxanthin (Anth), neoxanthin (Neo) or violaxanthin (Viola), and astaxanthin (AST). The chlorophyll pigments were as follows: pheophorbide a and b (Pheoa; Pheob), pheophytin a and b (Phya; Phyb), chlorophyll a, and b (Chla; Chlb). These results were consistent with the literature [28,29]. A complete list of TG molecular ions and pigments is also given in **Supplementary Table S3.1** and **Table S3.2**.

PCA analysis of polar lipid datasets from C-Auto or C-Hetero extracts showed discrimination between UAE, DCM and EtOH extracts, although some overlap of the 85% confidence curves was observed, being UAE and DCM closer (**Figure 3.2-A1** and **B1**). The first two dimensions (dim) capture 82.1 % and 71.3 % of the total variance in the C-Auto and C-Hetero extract datasets, respectively (**Figure 3.2-A1** and **B1**). Regarding C-Auto extracts, the sixteen lipid species that mainly contribute to the discrimination of the first dimension (Dim1) included 5 SQDG [SQDG(32:3), SQDG(32:2), SQDG(34:5), SQDG(34:4), SQDG(32:0)], 5 PI [PI(34:3), PI(36:5), PI(34:2), PI(35:2), PI(34:1)], 2 LPC [LPC(16:0), LPC(18:3)], 1 DGDG [DGDG(36:7)], 1 PG [PG(38:5)], 1 MGMG [MGMG(18:2)], 1 DGMG [DGMG(16:0)], all of which were less abundant in the EtOH extracts (**Supplementary Figure S3.3-A**). Regarding C-Hetero extracts, the lipid species that mainly contribute to the discrimination of the first principal component included 9 PI [PI(34:3), PI(36:5), PI(34:2), PI(35:2), PI(34:1), PI(32:2), PI(36:2), PI(36:3), PI(36:4)], 4 SQDG [SQDG(32:3), SQDG(30:0), SQDG(34:0), SQDG(34:4)], 1 MGMG [MGMG(16:0)], 1 DGDG [DGDG(28:0)], and 1 PC [PC(38:9)] (**Supplementary Figure S3.3-B**). These results show that in both cases, C-Auto and C-Hetero, the lipid species which contribute the most to the variation between the extraction methods are the polyunsaturated species of the PI and SQDG classes. Hierarchical cluster analysis showed greater similarity between DCM and UAE. The two groups clustered together at the first level of the tree and EtOH, forming its own meta-class, regardless of the conditions (**Figure 3.2-A2** and **B2**).

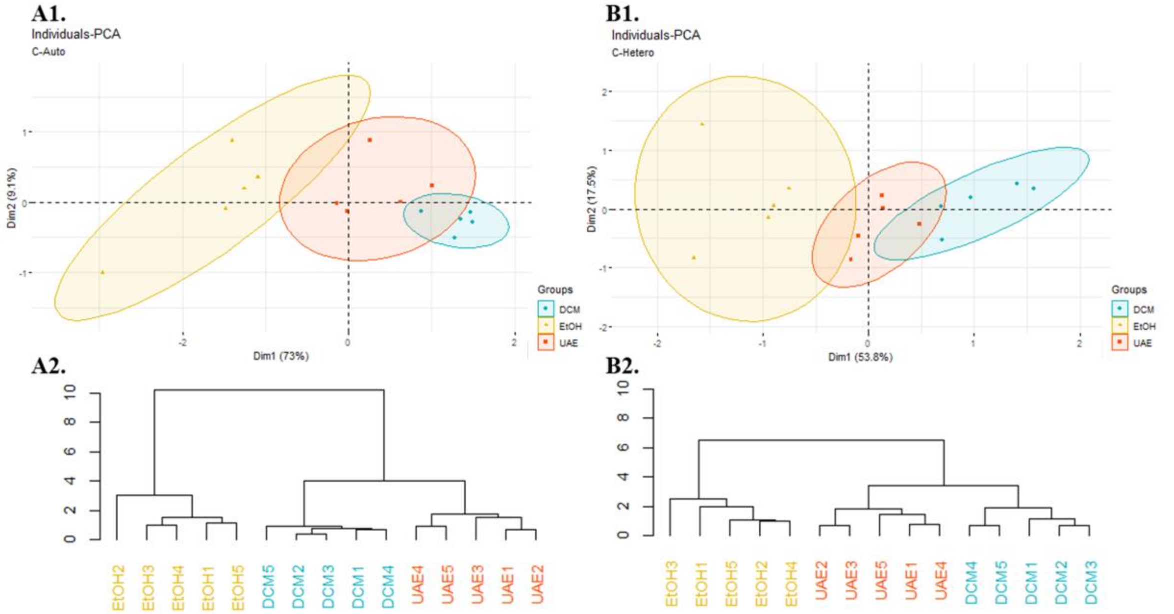

Figure 3.2. Principal component analysis score plot and hierarchical cluster analysis of the molecular ions of polar lipids identified in DCM, EtOH, and UAE extracts of C-Auto (A1; A2) and C-Hetero (B1; B2). Abbreviations: C-Auto, *C. vulgaris* cultivated under autotrophic conditions; C-Hetero, *C. vulgaris* cultivated under heterotrophic conditions; DCM, dichloromethane: methanol (2:1, v/v); EtOH, ethanol; UAE, ethanol extraction assisted with an ultrasound probe.

Univariate analysis of the C-Auto extracts (**Supplementary Table S3.3** and **Table S3.4**) revealed 122 of 153 significant differences between lipid species when comparing DCM and EtOH groups and when comparing DCM with UAE only eight lipid species were extracted differently. These species included 1 Cer [Cer(d35:0)], 3 DGDG [DGDG(35:1), DGDG(32:0), DGDG(32:5)], 1 MGDG [MGDG(32:3)], 2 PE [PE(30:0), PE(36:8)], and 1 PI_Cer [PI_Cer(t34:0-OH)], which were more abundant in the DCM group, except for the last two. Univariate analysis of the C-Hetero extracts **(Supplementary Table S3.5** and **Table S3.6**) also showed differences between the DCM and UAE groups, namely nine differences out of 41 significant lipid species and the species showing major variation belonging to the PC, MGDG, and PG classes. DCM and EtOH groups differed in 34 lipid species.

A heatmap (**Figure 3.3-A** and **B**) was plotted using the results of the univariate analysis and selecting a set of 40 lipid species with lower *p* values ($p < 0.05$) (**Supplementary Table S3.3** and **Table S3.5)**. In the case of the C-Auto extracts, the lipid species belonging to PE were more abundant in the UAE extract, being UAE > DCM >> EtOH. The species belonging to LPC, DGDG, MGDG, SQDG were more abundant in the DCM extracts, being DCM > UAE >> EtOH. In the C-Hetero extracts, most of the lipid species of all polar lipid classes were more abundant in the DCM extract, DCM > UAE >> EtOH. However, some PC species were more abundant in the EtOH extract than in the UAE extract.

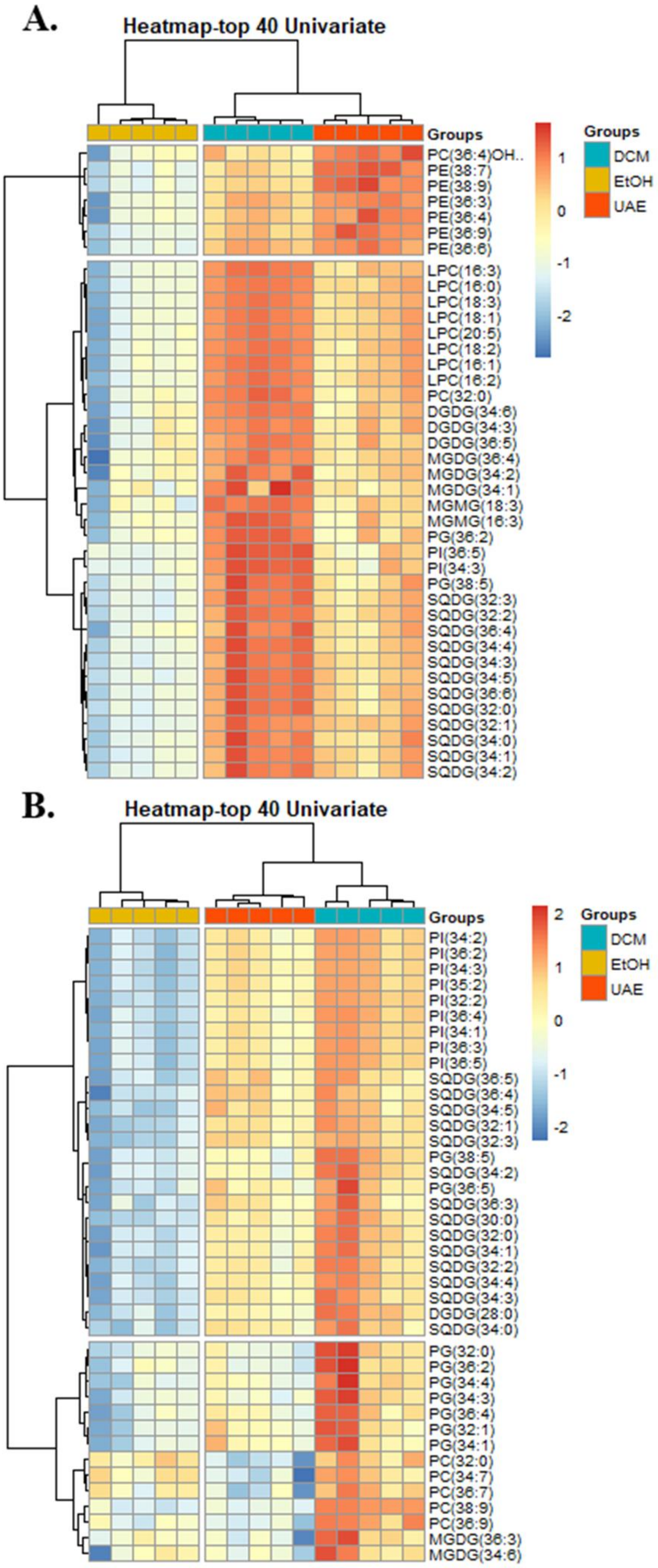

Figure 3.3. Two-dimensional hierarchical cluster heat map of the 40 most significant molecular ions (selected using univariate analysis, p-value < 0.05) of polar lipids extracted using different solvent

systems: (**A**) C-Auto samples and (**B**) C-Hetero samples. Quantity levels are shown on the colour scale, with numbers indicating the fold difference from the mean. The clustering of sample groups is represented by the dendrogram at the top. Abbreviations: C-Auto, *C. vulgaris* cultivated under autotrophic conditions; C-Hetero, *C. vulgaris* cultivated under heterotrophic conditions; DCM, dichloromethane: methanol (2:1, v/v); EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PG, phosphatidylglycerol; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; MGDG, Monogalactosyldiacylglycerol; LPC, lysophosphatidylcholine.

Statistical analysis was also carried out by comparing the TGs, and the pigments identified in the lipid extracts of C-Auto and C-Hetero obtained with different solvent systems. In the heatmap **(Figure 3.4)**, obtained using the 18 most discriminating species with lower *p* values from the Kruskal-Wallis test (*p*-value < 0.05), the abundance of the species was higher in the DCM extracts, with DCM >> UAE ≥ EtOH, regardless of culture conditions. In the case of the C-Auto samples, these species included 2 carotenoids [anth, neo or viola], 4 pigments [Chlb, pheoa, pheob, phya] and 12 highly unsaturated TGs [TG(52:2), TG(48:2), TG(48:3), TG(54:7), TG(52:8), TG(50:7), TG(52:7), TG(50:6), TG(50:5), TG(49:2), TG(53:5), TG(48:5)]. In C-Hetero, the low unsaturated TGs were the species that most contributed to discriminating DCM from other extracts. The dendrogram highlights the similarity between the EtOH and UAE extracts, the DCM extract forming its own meta-class **(Figure 3.4-A** and **B**).

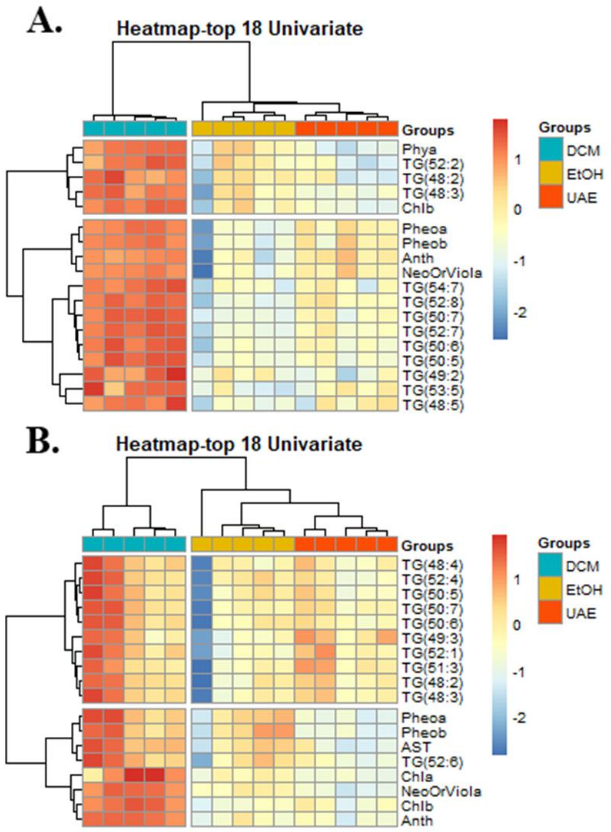

Figure 3.4. Two-dimensional hierarchical clustering heatmap of the 18 most significant species, selected using univariate analysis (*p*-value < 0.05), of neutral lipids and pigments discriminating the solvent systems used to extract total lipids: (**A**) C-Auto samples and (**B**) C-Hetero samples. Quantity levels are shown on the colour scale, with numbers indicating the fold difference from the mean. The dendrogram at the top represents the clustering of sample groups. Abbreviations: C-Auto, *C. vulgaris* cultivated under autotrophic conditions; C-Hetero, *C. vulgaris* cultivated under heterotrophic conditions; DCM, dichloromethane: methanol (2:1, v/v); EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe; Pheoa, pheophorbide a; Pheob, pheophorbide b; NeoOrViola, neoxanthin or violaxanthin; Anth, antheraxanthin; Phya, pheophytin a; Chlb, chlorophyll b; AST, astaxanthin; TG, triglycerides.

The results collected in the present work have shown that the use of an ultrasound probe to assist the ethanolic extraction was an effective method for improving the extraction yield of polar lipids from the biomass of *C. vulga*ris. Disruption of the cell wall of *C. vulgaris*, when subjected to UAE, resulted in higher lipid content with a similar composition of polar lipids compared to DCM extraction, the most effective method for the extraction of lipids.

Statistical analysis showed that the lipid extracts obtained with UAE had a similar composition of complex lipids (PLs and GLs) compared to the most efficient method for the extraction of lipid from *C. vulgaris* grown under C-Auto and C-Hetero conditions. Ethanolic extracts had lower extraction yield and different polar lipid composition compared to DCM, with lower extraction efficiency for anionic lipids such as PI, SQDG and PG. PI species are present in low abundance in membranes but have important regulatory roles as signalling molecules [30]. They are found in most intracellular compartments such as the endoplasmic reticulum (ER), Golgi apparatus, vacuoles, and plastids [30]. SQDG and PG species are mainly found in the thylakoid membranes of chloroplasts [17]. The intracellular localization of anionic lipids hinders their accessibility by EtOH. In this work, the EtOH, DCM and UAE extracts had the same lipid species, and no lipid decomposition or lipid oxidation was observed. This is consistent with one of the reported benefits of using ultrasonic extraction in which the quality of the extracted lipids is not compromised [31].

The UAE approach is a suitable method for providing extracts rich in polar lipids suitable for food and functional food applications. These extracts can be used as a source of important carriers of omega-3 and omega-6 fatty acids. These include phospholipids with ALA, such as PE(36:4), PE(36:6), LPC(18:3), PI(34:3), and PG(34:3), and LA, such as PG(36:2), PE(36:4), PI(34:3), LPC(18:2), PI(32:2), PI(34:2), PI(35:2), PI(36:3) (**Supplementary Table S3.7-Table S3.8** and **Figure S3.4-Figure S3.7**). These higher amounts of PLs with esterified omega-3 and omega-6 PUFAS in UAE extracts, compared to EtOH extracts contribute to its valorization as food ingredients. PLs are described as good suppliers of PUFAs to target organs such as the brain [32]. Indeed, omega-3 PLs supplementation appears to improve memory in elderly and neurological patients [32]. Additionally, some GLs esterified with ALA are also increased in UAE extracts compared to EtOH extracts, *e.g.*, MGMG(18:3), DGDG(34:3), DGDG(34:6), DGDG(36:5), SQDG(36:6) and SQDG(34:3) (**Supplementary Table S3.7-Table S3.8** and **Figure S3.4-Figure S3.7**). The DGDG and SQDG lipid species have been shown to have anti-inflammatory activities [17]. Thus, increasing the amount of SQDG polar lipid species in UAE extracts compared to EtOH extracts, and especially those containing bioactive omega-3 ALA and omega-6 LA fatty acids, provides healthier extracts for food formulations. In addition, the most abundant SQDG species of *C. vulgaris*, SQDG(32:0), which was found to be increased in UAE extracts compared to EtOH extracts, was also linked to desired biological activities such as antineoplastic activity [33].

In novel cuisine, the incorporation of *Chlorella* sp. biomass is a new trend due to their high content of bioactive compounds such as polyunsaturated fatty acids (PUFAs). However, the use of biomass directly in cooking raises some concerns such as fishy odour, unwanted flavour and strong green colour [34]. Thus, *C. vulgaris* extracts rich in omega-3 and -6 PUFAs are gaining interest as alternative ingredients for developing healthy and sustainable foods, with particular, but not exclusive interest in target populations such as vegans and vegetarians, the elderly, among others [3,9].

The polar lipids identified in the lipid extracts of *C. vulgaris*, especially those with ALA and LA, are valuable ingredients for novel functional products. The two essential FAs are not synthesized by humans and must be included in our daily diet. In the body, ingested ALA can be metabolized to bioactive docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), while LA can be metabolized to arachidonic acid, a precursor of prostaglandins. According to the FAO, ALA and LA should be included in the human diet at minimum daily intake values of 0.5 and 2.5 per cent of energy, respectively, to prevent symptoms of deficiency [35]. The ALA content of *C. vulgaris* can reach 27 % of total fatty acids [36]. Typical sources of ALA, such as soybeans and canola oil [35], have been reported with lower amounts of ALA, approximately 8 % and 12 % [37], respectively. In this work, the results showed that the relative percentage of ALA and LA in UAE lipid extracts was respectively 34 % and 20 % for C-Auto and 9 % and 34 % for C-Hetero and can therefore be used as a source of these essential fatty acids. Recent studies have shown that ALA and LA esterified to phospholipids and glycolipids are more absorbed when administered orally than TGs [32,38]. Dietary PLs can influence the FA composition of membrane phospholipids and signalling phospholipids and contribute to modulate several cell functions, depending on cell type and the ability to modulate membrane protein function [15]. The consumption of foods rich in polar lipids containing these essential FAs is beneficial for health and the prevention of diseases, such as inflammation, cardiovascular disease, cancer. It is also beneficial in the prevention of dyslipidemia, in particular for lowering total cholesterol and increasing high-density lipoprotein (HDL) cholesterol [15].

Extracts rich in PLs have broad roles in the food industry, such as emulsifiers, anti-spattering, and have gained increasing interest as antioxidant agents. Natural antioxidants, including extracts of microalgae, capable of neutralizing ROS, have been widely explored [39]. In this work, we examined the antioxidant activity of all extracts through their free radical scavenging potential against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radicals to compare the effectiveness of EtOH and UAE extracts for food industries.

3.5. The effects of different solvent systems on the antioxidant activity of lipid extracts of *Chlorella vulgaris*

The test of the DPPH radical scavenging activity of DCM extracts from C-Auto and C-Hetero was recently reported by our group [9]. In the present work, the bioactivity of DCM extracts was compared to that of EtOH and UAE extracts. Statistical differences were observed in the antioxidant activity between the DCM and UAE extracts of C-Auto at the highest concentration tested (q value < 0.05) (**Figure 3.5A**). Regarding the C-Hetero extracts, significant differences were detected between the DCM and UAE extracts at lower concentrations (12.5 and 62.5 µg/mL), the UAE exhibiting the highest activity (**Figure 3.5B**).

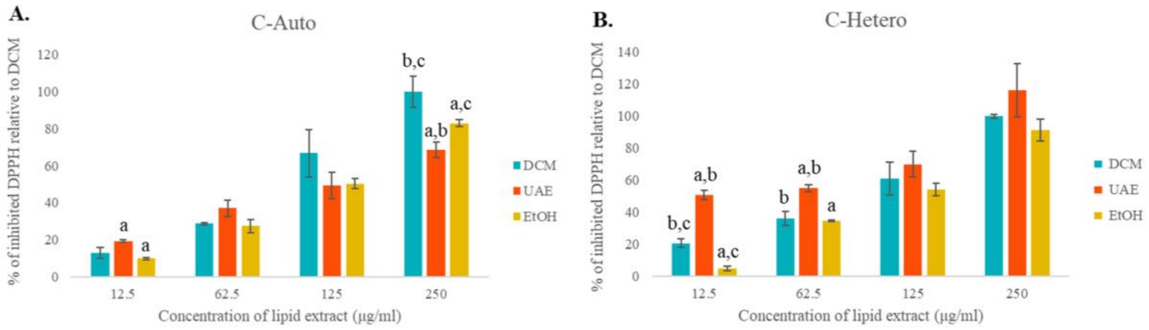

Figure 3.5. DPPH radical scavenging activity using lipid extracts obtained with different solvent systems (DCM, EtOH and UAE) of (**A**) *C. vulgaris* grown under autotrophic (C-Auto) and (**B**) heterotrophic (C-Hetero) conditions. The concentrations of the lipid extracts tested were 12.5, 62.5, 125 and 250 µg/mL. The results were expressed as percentages of inhibited DPPH relative to the control (the % inhibition with the DCM extracts at 250 µg/mL was considered to be 100%). Matching letters (a-c) indicate statistically significant differences between different solvent systems (Tukey's HSD post hoc analysis, $q < 0.05$).

Antioxidants in food products have been used to reduce deterioration and extend the shelf life of products [39]. Currently, extracts from edible and under-exploited microalgae species are considered a safe and economically feasible alternative to synthetic antioxidants [39]. In a previous study, Rodriguez-Garcia *et. al.* reported higher antioxidant activity for ethanolic extracts of *C. vulgaris* than synthetic antioxidants butylated hydroxyanisole and butylated hydroxytoluene [40]. In our work, antioxidant activity was observed for all the extracts of *C. vulgaris*. It should be noted that the differences observed in the amounts of lipids and pigments of the extracts did not significantly modify their potential antioxidant scavenging activity at a concentration of 125 µg/ml of lipid extract (**Figure 3.5A** and (**Figure 3.5B**). In addition, pigments, whose antioxidant properties have often been recognized, were observed in lower amounts in EtOH and UAE extracts, corroborating the contribution of other lipid compounds, such as GLs and PLs, to the antioxidant activity. PLs have been shown to inhibit the oxidation of their esterified n-3 PUFAS, better than TGs [15]. Overall, we can suggest that EtOH and UAE extracts of *C. vulgaris* are possible options for use as preservatives in the food industry.

4. Conclusions

Food ingredients or additives made from microalgae are recognized as safe by the Food and Drug Administration. Recently, the European Food Safety Authority has approved *C. vulgaris* as a food supplement and a food ingredient. Lipid extracts derived from microalgae are rich in omega-3 fatty acids and are a sustainable plant-based food ingredient alternative to fish oil, a limited resource and not suitable for vegetarians and vegans. Food grade extracts that use food-grade solvents like ethanol have low lipid extraction efficiency compared to chlorinated solvents which are more efficient but unsafe, hampering the marketing of these extracts as food ingredients.

This work evaluated the effects on the complex lipid and pigment composition of lipid extracts from two cultures of *C. vulgaris* under heterotrophic and autotrophic conditions extracted with food-grade solvent systems. By applying a lipidomic approach and identifying lipids at the molecular level, we have shown that by using a combination of ethanol assisted with UAE, we can obtain lipid extracts with a composition and properties similar to those obtained with more conventional and non-food grade systems. In addition, we have shown that *C. vulgaris* UAE extracts are rich in bioactive omega 3 phospholipids and glycolipids, capable of providing significant health benefits. These extracts can be a good alternative as food ingredients with health-promoting properties for the development of novel functional foods. In addition, the extracts obtained using ethanol assisted with UAE had significant antioxidant properties, being attractive as antioxidant additives in food products, as an organic ingredient replacing synthetic antioxidants and potentially increasing the shelf life of food products.

In conclusion, this work proposes an efficient lipid extraction method to recover polar lipids mainly esterified in linolenic from *C. vulgaris* cultivated in autotrophy or from extracts enriched in linoleic acid under heterotrophy suitable for food-grade applications. Ultrasound probe assisted ethanol extraction has provided promising lipid yields and extracts with higher lipid purity, reproducibility of polar lipid composition and richness in healthy omega 3 polar lipids for future applications in nutraceutical and food industries.

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CHAPTER 4 - Lipidomic signature and bioactive potential of new strains of *Chlorella vulgaris* (Honey and White *C. vulgaris*)

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Lipidomic signature and bioactive potential of new strains of *Chlorella vulgaris* (Honey and White *C. vulgaris*)

Abstract: Two mutants of microalga *Chlorella vulgaris* (abbreviated as C-Honey and C-White) developed by chemically-induced random mutagenesis have been reported as promising new foods due to their improved organoleptic and nutritional characteristics. This work aimed to assess fatty acid (FA) and polar lipid profiles of these new *C. vulgaris* mutants (GC-MS and C18-LC-MS), and screen for antioxidant and anti-inflammatory properties by in chemico assays (ABTS, DPPH and COX-2). C-Honey presented higher amounts of monounsaturated and omega-3 polyunsaturated FAs (PUFAs). C-White had higher amounts of saturated and omega-6 PUFAs. These results may explain the better antioxidant potential of C-Honey. LC-MS analysis allowed the identification of 211 lipid species, of which 207 were common in both *C. vulgaris* strains and 4 other betaine lipid species were only found in C-Honey. Major differences in the relative abundances of lipid species belonging to the phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and ceramide classes were noted. C-White had a greater abundance of oxidized phosphatidylethanolamines, which may be correlated with the better anti-inflammatory potential (34 ±1.5%). The results gathered in this work contribute to the valorization of the two mutants as new food supplements and ingredients with an interesting polar lipid profile with nutritional benefits for human health.

Keywords: Microalgae, Lipidomics, Polar lipids, *Chlorella vulgaris*, antioxidant activity, anti-inflammatory activity

1. Introduction

Microalgae are emerging ingredients with a wide diversity of nutrients and bioactive compounds. The consumption of products based on microalgae is increasing, which can be associated with their beneficial health properties, presenting themselves as great allies for a sustainable and healthy lifestyle. Currently, large-scale microalgal biomass production is considered one of the most promising strategies to meet the future needs of the next generations for food, feed, and pharmaceutical products [1].

Among commercialized microalgae, *C. vulgaris* has one of the highest market values, expected to reach $412.3 million by 2028, and an actual yield of 5,000 tons of dry matter per year [2]. The boost in the exploitation of this microalga is due to the fact that it can be cultivated in different conditions, thus allowing the reduction of production costs, and also contributing to the development of specific strains. The most common growth condition is the autotrophic growth [3], which produces autotrophic *C. vulgaris* (C-Auto). Currently, a cost-effective method of *C. vulgaris* grown heterotrophically has been established, resulting in heterotrophic *C. vulgaris* (C-Hetero) [1]. The main application of *C. vulgaris* strains is in the food and feed industries, but also in the cosmetic and pharmaceutical industries, among others as reviewed in [4]. However, their organoleptic properties such as colour, taste and odour may limit its application in foods containing microalgal biomass [5]. Thus, the improvement of these organoleptic properties is of the utmost importance. Therefore, two new strains were developed from the C-Hetero strain by selective breeding [6]. These new strains, called White and Honey *C. vulgaris,* are chlorophyll-deficient mutants of *C. vulgaris*, characterized by a light white and yellow colour, respectively [6]. These two new strains also exhibit less intense odour and improved texture and taste, making them more attractive for use in food products [6]. C-Honey and C-White are already recognized as safe and approved for human consumption by the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA). These microalgae can be used as functional ingredients and in the development of innovative foods.

C. vulgaris contains high levels of protein and lipids and is therefore exploited for several nutritional and industrial purposes [7,8]. The mutagenesis that gave rise to the two new *C. vulgaris* strains seem to alter the content in these important compounds. For instance, both C-Honey and C-White are rich in protein, with reported values of 39.5% dry weight (DW) in C-Honey and 48.7% DW in C-White, representing an increase of 12% and 38% compared to the wild strain (C-Auto) [6]. Concerning the carotenoid content, C-Honey retains an alluring lutein content, unlike C-White which has no carotenoid apart from the colourless phytoene [6]. However, little is known about their detailed lipid composition, such as their fatty acid (FA) and lipid profile.

C. vulgaris has a high lipid content between the range of 5 to 68% dry weight [4]. The C-Auto (wild type strain) and C-Hetero strains of *C. vulgaris* have already been characterized for their lipid composition, showing an interesting fatty acid profile, being abundant in omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) [9–11]. Their polar lipidome is rich in several bioactive phospholipids and glycolipids esterified with C18:2 *n*-6 and C18:3 *n*-3 [9]. For this reason, several beneficial properties have been described for these strains, such as antioxidant and anti-inflammatory effects [9,10]. It is known that *C. vulgaris* is chemoplastic and its polar lipid profile is modulated according to growth conditions [9]. Therefore, it is also important to evaluate the lipid profile of these new strains (C-Honey and C-White), which should also have different lipid profiles depending on growth conditions and therefore different nutrients and to assess their bioactive properties.

Thus, this study aims to characterize the FA and polar lipidome signature of the *C. vulgaris* strains C-Honey and C-White, and bioprospecting the anti-inflammatory and antioxidant activities of their lipid extracts to evaluate their potential as functional food ingredients and therapeutical alternatives.

2. Materials and methods

2.1. Reagents

HPLC grade dichloromethane (CH_2Cl_2), absolute ethanol 96% (CH_3CH_2OH), and methanol (CH_3OH) were purchased from Fisher Scientific Ltd. (Loughborough, UK). The water was of Milli-Q purity (Synergy1, Millipore Corporation, Billerica, MA, USA). The 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet +}$) was obtained from Fluka (Buchs, Switzerland) and the α,α-diphenyl-β-pic-rylhydrazyl radical ($DPPH^{\bullet}$) was purchased from Aldrich (Milwaukee, WI, USA). All other reagents were purchased from major commercial sources. Lipid internal standards were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA): 1,2-dimyristoyl-*sn*-glycero-3-phospho-(10-rac-glycerol) (dMPG), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (dMPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylinositol (dMPI), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (dMPE), 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylserine (dMPS), 1-nonadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC), N-heptadecanoyl-D-*erythro*-sphingosine (Cer), and 1',3'-bis-[1-2-di-tetradecanoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (CL).

2.2. Microalgae strain and culture media

Chlorella vulgaris strain (AGF002) was obtained from Allmicroalgae culture collection.

Heterotrophic *C. vulgaris* has been produced in Allmicroalgae facilities as reported in previous work [1,9]. Briefly, the heterotrophic culture was grown in Guillard’s F2 culture medium adapted to local water using nitrate and glucose with a C:N ratio of 6.7:1. This culture was obtained sequentially from 50-250 mL Erlenmeyer flask to 5 L bench-top fermenter (New Brunswick BioFlo® CelliGen®115; Eppendorf AG, Hamburg, Germany), and later to 200 L and 5000 L industrial fermenters. All fermenters were operated in fed-batch under controlled temperature, pH and dissolved oxygen. Chlorophyll-deficient mutants of *C. vulgaris* have been obtained by chemically induced random mutagenesis from the heterotrophic *C. vulgaris*, as described in [6]. Briefly, *C.*

vulgaris cells were cultured and treated with different concentrations of ethyl methanesulfonate (EMS, Merck, USA) for 1 h in the dark. A colony of *C. vulgaris* mutants coloured yellow (C-Honey) was selected by visual observation of the plates in dim light, isolated and sub-cultured several times on count agar plates (VWR, Portugal). Then, C-Honey was grown to an exponential phase and subjected to a second round of random mutagenesis with 300 mM EMS. This time, a colony with white colour (C-White) was selected and sub-cultured on count agar plates with 10µM norflurazon (a metabolic inhibitor of carotenoids biosynthesis) and incubated at 30 ºC in the dark for 1 week. C-White colonies were subcultured multiple times, with and without norflurazon to confirm mutant phenotypic stability.

Mutant strains were considered stable after 50 generations expressing the same phenotype. To further ensure mutation stability, stock cultures of the mutants were maintained with the same inhibitor (10 µM norflurazon), to maintain the selective pressure used for the initial mutagenesis process and thus prevent reversion of the mutation.

2.3. Lipid extraction

Total lipid extraction was performed according to Folch’s method [12], with some modifications described here [9]. Folch’s method was used to extract lipids from the two mutant strains of *C. vulgaris*, as it was shown to be more efficient, compared to the Bligh and Dyer’s method, in extracting lipids from microalgae [13]. This method has also been used in the study of the lipidome of autotrophic (wild-type) and heterotrophic *C. vulgaris* strains [9]. The extraction was carried out individually for each strain (n = 5), from 25 mg of lyophilized biomass using a solvent mixture of CH_2Cl_2:CH_3OH (2:1, v/v). The suspension was centrifuged at 2000 rpm for 10 min (Selecta JP Mixtasel, Abrera, Barcelona, Spain) and the supernatants were collected and dried under a stream of nitrogen. This step was repeated 3 times. Then, the dried extracts were re-extracted with 2 mL of CH_2Cl_2, 1 mL of CH_3OH and 0.75 mL of Milli-Q water, centrifuged (2000 rpm for 10 min) and the lipid-containing layers were collected and dried. The aqueous phase was re-extracted with 2 mL of CH_2Cl_2 two more times. The extracts obtained were weighed and the extraction yield was calculated as a percentage of dry weight (DW), with the following equation (1):

$$Lipid\ yield\ \left(\%DW,\frac{w}{w}\right)=\frac{Weight\ of\ lipid\ extract\ (mg)}{Weight\ of\ biomass\ (mg)}x100\ (1)$$

2.4. Fatty Acid Analysis

Analysis of the fatty acid profile of the C-Honey and C-White extracts was carried out by gas chromatography-mass spectrometry (GC-MS). Fatty acid methyl esters (FAMEs) were prepared from 60 µg of lipid extracts as described [14,15]. A solution of methyl nonadecanoate (internal standard) at 0.96 µg mL^{-1} was added to the FAMEs solution (Sigma, St. Louis, MO, USA). Analyses were performed on an Agilent Technologies 8860 GC System (Santa Clara, CA, USA) interfaced with an Agilent 5977B Mass Selective Detector (Agilent, Santa Clara, CA, USA) with electron impact ionization (70 eV) and scanning the range of *m/z* 50–550 in a 1s cycle using full scan mode acquisition. A DB-FFAP capillary column (30 m long x 0.32 mm internal diameter, 0.25 µm film thickness (J&W Scientific, Folsom, CA, USA)) was used. The following conditions were used during the chromatographic analysis: injection volume 2 µL (splitless), a constant flow rate of 1.4 mL min^{-1} of helium gas, inlet temperature 230 °C, and detector temperature 220 °C. The oven temperature was programmed as follows: 58 °C for 2 min, 25 °C min^{-1} to 160 °C, 2 °C min^{-1} to 210 °C, 30 °C min^{-1} to 225 °C (held for 10 min). The data acquisition software used was GCMS5977B/Enhanced MassHunter. Agilent MassHunter Qualitative Analysis 10.0 software, NIST library and literature were used to identify FAs. The relative abundance (RA) of fatty acids was obtained by normalizing the data with the internal standard.

Finally, five lipidic quality indices were determined. The atherogenic (AI), thrombogenic (TI) and hypocholesterolemic/ hypercholesterolemic indeces (h/H) were calculated as proposed by Ulbricht and Southgate [16], with equations 2,3 and 4, respectively. The nutritive value (NVI) and the peroxidation index value (PI) were determined according to [17,18], with equations 5 and 6 respectively.

$$AI=\frac{[C12{:}0+(4*C14{:}0)+C16{:}0]}{[\sum MUFA+\sum(n\text{-}6)+\sum(n\text{-}3)]}\ (2)$$

$$TI=\frac{[C14{:}0+C16{:}0+C18{:}0]}{[(0.5*\sum MUFA)+(0.5*\sum(n\text{-}6))+(3*\sum(n\text{-}3))+(\frac{\sum(n\text{-}3)}{\sum(n\text{-}6)})]}\ (3)$$

$$(\mathrm{h/H}) = \frac{[\text{cis-C18:1}+\sum\text{PUFA}]}{[\text{C12:0}+\text{C14:0}+\text{C16:0}]}\ (4)$$

$$NVI = \frac{C18{:}0 + C18{:}1}{C16{:}0}\ (5)$$

$$PI = (monoenoic\ acid * 0.025) + (dienoic\ acid * 1) + (\text{trienoic acid} * 2) + (\text{tetraenoic acid} * 4) + (\text{pentaenoic acid} * 6) + (\text{hexaenoic acid} * 8)\ (6)$$

2.5. C18- Liquid chromatography-mass spectrometry (C18–LC–MS)

Lipid extracts were analysed by reverse phase liquid chromatography in a Dionex Ultimate 3000 (Thermo Fisher Scientific, Bremen, Germany) using an Ascentis® Express 90 Å C18 column (Sigma-Aldrich®, 2.1 x 150 mm, 2.7 µm) coupled to the Q-Exactive® hybrid quadrupole Orbitrap mass spectrometer (Thermo Fisher, Scientific, Bremen, Germany). Mobile phase A was composed of water/acetonitrile (40/60%) with 10 mM ammonium formate and 0.1% formic acid. Mobile phase B was composed of isopropanol/acetonitrile (90/10%) with 10 mM ammonium formate and 0.1% formic acid.

The following gradient was applied: 32% B at 0 min, 45% B at 1.5 min, 52% B at 4 min, 58% B at 5 min, 66% B at 8 min, 70% B at 11 min, 85% B at 14 min, 97% B at 18 min, 97% B at 25 min, 32% B at 25.01 min and 32% B at 33 min.

For this analysis, a volume of 5 µL of a mixture containing 40 µg of lipid extract from each sample dissolved in 20 µL of dichloromethane, 72 µL of a solvent system consisting of 50% isopropanol/ 50% methanol, and 8 µL of a mixture of phospholipid standards (dMPC - 0.04 µg, SM d18:1/17:0 - 0.04 µg, dMPE - 0.04 µg, LPC - 0.04 µg, dPPI - 0.08 µg, CL(14:0)4 - 0.16 µg; dMPG - 0.024 µg, Cer(17:0/d18:1) - 0.08 µg, dMPS - 0.08 µg; dMPA – 0.16 µg), was loaded into the column at 50 °C and at a flow-rate of 260 µL min^{-1}.

The mass spectrometer was operated in simultaneous positive (ESI 3.0 kV) and negative (ESI −2.7 kV) modes as previously described. The capillary temperature was 320 °C and the sheath gas flow was 35 U. Data was acquired in full scan mode with a high resolution of 70,000, automatic gain control (AGC) target of 3 x 10^6, in an *m/z* range of 300-1600, 2 micro scans, and maximum injection time (IT) of 100 ms. Tandem mass spectra (MS/MS) were obtained with a resolution of 17,500, AGC target of 1x10^5, 1 micro scan, and maximum IT of 100 ms. The cycles consisted of a full-scan mass spectrum and ten data-dependent MS/MS scans, which were repeated continuously throughout the experiments with a dynamic exclusion of 30 s and an intensity threshold of 8 x 10^4. The normalized collision energy (CE) ranged between 20, 24 and 28 eV in the negative mode and 25 and 30 eV in the positive mode. Data acquisition was performed using the Xcalibur data system (V3.3, Thermo Fisher Scientific, Bremen, Germany).

Lipid species were identified using mass spectrometry-data independent analysis (MS-DIAL) v4.70 software and manual data analysis [19–21], and integrated in the MZmine *v*2.53 software [22]. The areas of the peaks of each lipid species were normalized by calculating the ratio against the area of the selected internal lipid standard (with the closest retention time). The relative abundance of each lipid species was estimated by dividing the normalized peak areas of each lipid species by the sum of the total normalized peak areas.

2.6. DPPH Radical Scavenging Assay and ABTS radical cation scavenging assay

The antioxidant activity of the lipid extracts from C-Honey and C-White against the α,α-diphenyl-β-pic-rylhydrazyl radical ($DPPH^{\bullet}$) and the 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) was evaluated as previously reported [9]. Briefly, a volume of 150 μL of an ethanolic dilution of the lipid extracts (25, 125, 250, 500 μg mL^{-1}) were mixed with 150 μL of $DPPH^{\bullet}$ (or $ABTS^{\bullet+}$) working solution in ethanol (abs~0.9). The samples were incubated for 120 min and the absorbance was measured at 517 nm (or 734 nm for $ABTS^{\bullet+}$) every 5 min using a UV-vis spectrophotometer (Multiskan GO 1.00.38, Thermo Scientific, Hudson, NH, USA). Controls were prepared by replacing the radical solution with ethanol. To monitor the radical stability solutions with the radical plus ethanol were prepared. All the measurements were carried out in triplicate.

The same procedure was applied to the Trolox standard solution (25, 125, 250, 500 μg mL^{-1} in ethanol). The antioxidant activity was obtained using Eq. (7), and is expressed as the percentage of inhibition of the $DPPH^{\bullet}$ (or $ABTS^{\bullet+}$):

$$\text{Inhibition } \% = \frac{(\text{AbsRadical} - (\text{AbsSample} - \text{AbsControl}))}{\text{AbsRadical}} \text{x}100 \ (7)$$

Abbreviations: AbsRadical, absorbance of radical (DPPH$^{\bullet}$ or ABTS$^{\bullet+}$); AbsSample, absorbance of the sample with radical (DPPH$^{\bullet}$ or ABTS$^{\bullet+}$); AbsControl, absorbance of the sample with ethanol

The activity expressed in Trolox Equivalents (TE) was calculated using Eq. (8), where the IC10 values are the concentration of the sample or of Trolox, which induces the reduction of the DPPH$^{\bullet}$ (or ABTS$^{\bullet+}$) radical in 10 %:

$$\text{TE } (\mu\text{mol/g}) = \frac{\text{IC 10 Trolox } (\mu\text{mol/g})}{\text{IC 10 of samples } (\mu\text{g/mL})} \text{x}1000 \ (8)$$

2.7. Anti-inflammatory activity

The anti-inflammatory activity of the lipid extracts from C-Honey and C-White *C. vulgaris* was evaluated with the cyclooxygenase-2 (COX-2) inhibition assay. This assay was carried out using the commercial COX-2 inhibitory screening assay kit—Cayman test kit-701080 (Cayman Chemical Company, Ann Arbor, MI, USA). Briefly, lipid extracts were dissolved in 100% DMSO to a final concentration of 20, 60 and 125 μg mL^{-1}. The amount of prostaglandin F2α produced was quantified by spectrophotometry (415 nm, Multiskan GO 1.00.38, Thermo Scientific, Hudson, NH, USA) and processed with the software SkanIT version 3.2 (Thermo Scientific). The results were expressed as a percentage of inhibited COX-2.

2.8. Statistical analysis

Statistical analyses were performed using R version 4.0.2 [23] in Rstudio version 1.3.1093 [24]. All data were log-transformed. The assumptions of normality and homogeneity of variance were verified by *Shapiro-Wilks* and *Levene* tests, respectively. All data were not normally distributed, and the variances were not equal. Thus, the *Wilcoxon rank-sum test* was performed to compare all datasets (fatty acid, polar lipid profile and lipid classes). The Principal component analysis (PCA) and the ellipses were created using the R libraries FactoMineR [25] and factoextra [26]. The *Shapiro-Wilk* and *Levene* tests were performed using the R package RVAideMemoire [27] and Car [28], respectively. The *Wilcoxon rank-sum* test was performed using the R package rstatix [29]. *P*-values were corrected for multiple testing using the BH Benjamini, Hochberg, and Yekutieli method (*q*-values) [30]. A *q*-value < 0.05 was considered an indicator of statistical significance. Heatmaps and Hierarchical Cluster Analysis (HCA) were created using the R package pheatmap using “Euclidean” as clustering distance and “ward.D” as the clustering method [31]. The heatmap was constructed based on the 50 polar lipid species with the lowest *p*-values ($p < 0.05$) in *Welch's t-test*. Boxplots and graph bars were created using the R package ggplot2 [32].

3. Results and discussion

3.1. Lipid content of Honey and White *C. vulgaris*

The lipid content (mg/100 mg of biomass dry weight (DW)) of Honey and White C. vulgaris was 5.0 ± 1.2 % and 4.8 ± 0.5 %, respectively. The lipid content of Honey and White C. vulgaris was similar and very close to the lower limit of values reported for C. vulgaris [4]. Several published works reported the lipid content of *C. vulgaris* and the results are very diverse [9,33–38]. For example, Conde and co-workers reported a lipid content of 8.8 % for *C. vulgaris* [34], while Madkour reported 26.7 % [35]. These differences may be due to the culture conditions and the lipid extraction methodology used. Additionally, there are studies evaluating the effects of the culture conditions on the lipid production of *C. vulgaris* [36,39]. For example, it has been observed that an increase in CO_2 supply from 2 to 10 % induced a decrease in the lipid content of *C. vulgaris* from 43.0 % to 32.8 % [37]. Temperature changes also affect the lipid production of *C. vulgaris*; for example, an in-crease from 25 to 30 ºC induced a decrease in lipid content from 14.7 % to 5.9 % [38].

Thus, and in comparison, with the literature, the two mutant strains of *C. vulgaris* showed lower lipid yield compared to *C. vulgaris* grown under autotrophic (wild type) and heterotrophic conditions (12.5 ± 0.9 % and 7.9 ± 0.7 %, respectively) (data shown in **Supplementary Table S4.1**) [9]. These data indicate that culture conditions and different strains of *C. vulgaris* appear to influence the different abilities to accumulate lipids. Moreover, it is important to point out that the two mutants have lower chlorophyll content [6], which may contribute to the lower lipid levels observed in these strains since the pigments are liposoluble compounds and are found in the lipid extracts. Pigments, on the other hand, may also contribute to the higher lipid levels reported in C-Auto and C-Hetero (with high content of pigments) [40]. In addition, it has also been pointed out that random mutagenesis can have pleiotropic effects, affecting several untargeted genes, namely encoding for enzymes involved in lipid metabolism, which can lead to unexpected characteristics of mutants such as a reduced lipid content [41].

However, compared to other *Chlorella* species (*Chlorella pyrenoidosa*) and food matrices (soybean and wheat) C-Honey and C-White have a high lipid composition [42], and thus these new strains may be useful as a new food and food ingredients.

3.2. Fatty acid profile of Honey and White *C. vulgaris*

The FA profile of Honey and White *C. vulgaris* was determined by GC-MS analysis of FAMEs. This analysis allowed the identification and quantification of fourteen FAs **(Table 4.1)**, similar to those previously reported for *Chlorella vulgaris* [9,43,44].

Table 4.1. GC-MS analysis of the fatty acid composition of total lipid extracts of *C. vulgaris* Honey (C-Honey) and C. vulgaris White (C-White). Data are presented as mean ± standard deviation (SD) (n = 5). The most abundant FAs present in C-Honey and C-White are highlighted in bold.

Fatty acid	**C-Honey**	**C-White**	***q* values**
C14:0	0.2±0.0	0.2±0.0	*
C15:0	0.1±0.0	0.1±0.0	-
C16:0	**24.7±0.4**	**26.3±0.6**	*****
C16:1(*n*-9)	2.7±0.1	2.8±0.2	-
C16:1(*n*-7)	0.2±0.0	0.1±0.0	*
C16:2(*n*-6)	3.8±0.2	8.6±0.6	*
C17:0	0.8±1.0	0.2±0.0	*
C16:3(*n*-3)	4.7±0.3	1.0±0.1	*
C18:0	**9.6±0.4**	**12.7±3.4**	**-**
C18:1(*n*-9)	**18.0±0.2**	**15.3±0.8**	*****
C18:1(*n*-7)	0.4±0.0	0.3±0.1	*
C18:2(*n*-6)	**25.5±0.3**	**30.5±1.8**	*****
C18:3(*n*-3)	9.0±0.2	1.8±0.1	*
C20:0	0.4±0.0	0.2±0.1	*
Σ SFA	35.0±1.1	39.7±3.4	*
Σ MUFA	20.9±0.3	18.4±1.0	*
Σ PUFA	44.1±0.9	41.9±2.5	-
Σ (*n*-3)	**14.3±0.5**	**2.8±0.3**	*
Σ (*n*-6)	**29.8±0.5**	**39.0±2.3**	*****

Abbreviations: ∑ PUFA, the sum of polyunsaturated fatty acids; ∑ MUFA, the sum of monounsaturated fatty acids; ∑ SFA, the sum of saturated fatty acids; *Statistically significant differences between mutants of *C. vulgaris* (Wilcoxon rank sum test, q < 0.05).

The same FAs have been identified in C-Honey and C-White but significant differences in their amounts can be highlighted. Of the fourteen FAs identified, eleven were significantly different (*Wilcoxon rank sum test*, $q < 0.05$) between the two groups (**Supplementary Table S4.2**). The most abundant FAs found in C-Honey and C-White were C18:2 (*n*-6) (linoleic acid; LA), C16:0, C18:1(*n*-9) and C18:0 (**Supplementary Figure S4.1**). C-White had a greater abundance of omega-6 polyunsaturated FAs (PUFAs) than C-Honey, especially the C18:2(*n*-6) (linoleic Acid, LA), while C-Honey has a greater content of omega-3 PUFAs, namely C16:3(*n*-3) and C18:3(*n*-3) (alpha-linolenic acid; ALA).

A comparison of our results with the literature revealed that C-Honey had higher levels of omega-3 FAs compared to C-Hetero (14.3 ± 0.5 % *vs* 10.4 ± 0.6 %), but a lower content compared to the wild-type C-Auto (14.3 ± 0.5 % *vs* 36.1 ± 2.2 %) [9]. C-Auto is by far the microalgae with the highest relative abundance of omega-3 PUFAs (C16:3(*n*-3) and C18:3(*n*-3)) and lower amounts of C16:0 and C18:2(*n*-6). Data collected in this work and obtained from the literature revealed a FA signature specific to the *C. vulgaris* strains, with C-Honey showing similarities to the C-Hetero strain, while C-Auto and C-White exhibit a more different FA profile [4]. The mutants of *C. vulgaris* evaluated in this work showed reduced content of ALA and higher levels of LA compared to *C. vulgaris* grown under autotrophic conditions [9]. Lipid extracts rich in these FAs are of utmost importance for developing novel functional foods based on *Chlorella*. These essential FAs cannot be synthesized by humans and must be provided by food in an appropriate proportion. The nutraceutical value of these unsaturated FAs isolated from *Chlorella* strains has already been reported with beneficial effects on hyperglycemia, hyperlipidemia, hepatic steatosis, and adipocyte hypertrophy in mice fed a rich fat diet [45].

Both C-Honey and C-White showed higher values of LA compared to other microalgae. Of the 63 species of microalgae summarized in a review paper [46], only two (*Arthrospira platensis* D880 and *Chlamydomonas mexicana*) were reported with higher levels of C18:2(*n*-6) compared to C-Honey and C-White. Moreover, of the seven commercialized microalgae (*Chlorella vulgaris*, *Chlorococcum amblystomatis*, *Scenedesmus obliquus*, *Tetraselmis chuii*, *Phaeodactylum tricornutum*, *Spirulina* sp. and *Nannochloropsis oceanica*), all had lower amounts of these FAs [34]. Additionally, compared to other major sources of LA (canola oil) [47], C-Honey and C-White have higher amounts of this PUFA.

These results suggest that the two new strains of *C. vulgaris* are a source of LA-rich functional lipid extracts, which can be applied as promising new ingredients in the development of supplements and foods. Despite the potential of the lipid extracts, we also wanted to assess the potential of the whole biomass of the mutants as PUFAs supplements compared to wild-type biomass. The results show that by measuring the quantity (in µg) of fatty acids per mg of dry biomass (**Supplementary Table S4.3**), the wild type will be a better supplement of PUFAs since it is richer in % of dry biomass (approximately 3.2 % against 1.9 % for C-Honey and 0.6 % for C-White). However, mutant strains are distinguished by high protein content, making them a better resource for protein supplements [6].

To predict the potential nutritional and health benefits of the lipid extracts of C-Honey and C-White, five nutritional quality indices were determined: the atherogenic index (AI), the thrombogenic index (TI), hypocholesterolemic/hypercholesterolemic (h/H), nutritive value (NVI) and peroxidation index value (PI) (Table 4.2).

Table 4.2. Lipidic quality indexes of total lipid extracts of *Chlorella vulgaris* Honey (C-Honey) and *Chlorella vulgari*s White (C-White). Data are means ± SD, n = 5.

Indexes	C-Honey	C-White	***q* value**
***n*-6/*n*-3 ratio**	2.1±0.0	13.9±0.6	*
AI	0.4±0.0	0.5±0.0	*
TI	0.5±0.0	1.1±0.2	*
h/H	2.5±0.0	2.2±0.1	*
NVI	1.1±0.0	1.1±0.1	-
PI	58.9±1.4	45.1±2.7	*

Abbreviations: h/H ratio, hypocholesterolemic/hypercholesterolemic indices; AI, atherogenic index; TI, thrombogenic index; NVI, nutritive value index; PI, peroxidation index; SD, standard deviation; *Statistically significant differences between mutants of *C. vulgaris* (*Wilcoxon rank sum test*, $q < 0.05$).

Significant differences between C-Honey and C-White were observed for the *n*-6/*n*-3 ratio, the AI, h/H, TI and PI indexes, while the NVI index was similar in both strains of *C. vulgaris*. C-Honey had the lowest *n*-6/*n*-3 ratio (2.1±0.0), AI value (0.4±0.0) and TI value (0.5±0.0), while C-White had the lowest h/H ratio (2.2±0.1) and PI value (45.1±2.7).

The use of lipidic quality indexes has already been described in the literature to assess the health benefits of lipids from different food matrixes. The AI and TI are predictors of cardiovascular disease risk, which measure the probability of reducing atherogenic plaques and blood clots formation, respectively [34,48]. Lower values of AI and TI are associated with a higher protective effect against cardiovascular disease [49]. Both strains evaluated show beneficial cardiovascular properties, with low values for AI (0.4±0.0 for C-Honey *versus* 0.5±0.0 for C-White) and TI (0.5±0.0 for C-Honey *versus* 1.1±0.2 for C-White). Comparing these indexes with those previously reported for *C. vulgaris* (0.2±0.0) [34], these new strains had higher values of AI and TI. The values collected in this work were also similar to values previously reported for other microalgae such as *Scenedesmus obliquus* and also *Spirulina* sp. [34], which is approved for human consumption and considered a superfood [50,51]. The NVI of the lipid extracts of C-White and C-Honey was also evaluated, and due to higher levels of palmitic acid (C16:0), C-Honey and C-White exhibit high values for NVI compared to other algae, such as *Grateloupia turuturu* [18]. The NVI is often used as an indicator of the effect of FAs on the metabolism of cholesterol, with higher values being the most beneficial to human health [52]. In addition, the stability of PUFAs was evaluated by calculating the PI [18]. C-White obtained the lowest PI value, indicating that it is less susceptible to oxidation compared to C-Honey, explained with the higher proportions of MUFAs and PUFAs in C-Honey.

C-Honey and C-White have different FA profiles, with significant changes in omega-3 (14.3±0.5 *vs.* 2.8±0.3) and omega-6 (29.8±0.5 *vs.* 39.0±2.3) PUFAs content, directly correlated with the values obtained for the *n*-6/*n*-3 ratio (2.1±0.0 *versus* 13.9±0.6). In conclusion, the index values reported in this study for C-Honey and C-White show that both strains are characterized by good nutritional indicators, thus making these strains a valuable source of nutrients to introduce into a healthy diet, among other applications [34]. Regarding the measured indices and FA profiles, our results indicate that C-Honey is more desirable in terms of potential health benefits while C-White appears to be less prone to oxidation, suggesting better stability as a food ingredient.

3.3. The polar lipid signature of Honey and White *C. vulgaris*

The polar lipidome of White and Honey *C. vulgaris* was analyzed by high-resolution C18 LC-MS and MS/MS. In this analysis, we identified and quantified 207 lipid species in C-White and 211 in C-Honey (Table 4.3**; Supplementary Figure S4.2-S4.15 and Supplementary Table S4.4**). The identified lipid species belonged to 4 different classes of glycolipids [monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG) and diacylglycerylglucuronide (DGGA)], 8 classes of phospholipids (PL) [phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylinositols (PI), phosphatidylglycerols (PG), phosphatidylserines (PS), cardiolipins (CL), lysophosphatidylcholines (LPC) and lysophosphatidylethanolamines (LPE)], 1 class of betaine lipids (BL) [diacylglyceryltrimethylhomoserine (DGTS)] and 1 class of sphingolipids [ceramides (Cer)]. Furthermore, in some classes of lipids, oxidized lipid species such as hydroxy (PE-OH, Cer-OH) and hydroperoxides derivatives (PE-2O and PI-2O) have also been found.

Lipidomic analysis showed that C-White and C-Honey exhibit a similar lipid signature, as they are composed of the same lipid classes and with 207 lipid species in common. The only differences between the two microalgae were in the total number of DGTS species, of which 4 DGTS (32:2, 34:2, 35:2 and 36:4) were only detected in C-Honey. Most of the lipid classes found in the new strains of *C. vulgaris* have already been identified in the lipidome of *C. vulgaris* [9–11]. However, in this analysis, we identified three new classes of lipids (DGGA, DGTS, and CL), never reported in *C. vulgaris*. Moreover, in this study, we were able to identify 96 lipid species in C-Honey (2 CL, 1 DGGA, 10 MGDG, 22 PE, 11 PG, 11 PI, 17 Cer, 9 DGDG, 8 DGTS, 4 PC and 1 PS) and 92 in C-White (2 CL, 1 DGGA, 10 MGDG, 22 PE, 11 PG, 11 PI, 17 Cer, 9 DGDG, 4 DGTS, 4 PC and 1 PS), which have not been identified in previous studies [9,10] (**Supplementary Table S4.4**). This may be due to differences in the lipid metabolism of the two strains, although it cannot be excluded that some differences may be due to the different analytical methodology since the acquisition of the lipidome data in the present work was performed by reverse phase C18 LC-MS and identification with MS-DIAL software, whereas in previous works, data were acquired by HILIC LC-MS and manual identification lipid species.

Our results show that PC and PE species were the most abundant classes in both C-White and C-Honey, indicating that these microalgae are particularly rich in phospholipids, consistent with what has been previously reported for the strains of *C. vulgaris* [9]. LC-MS analysis also showed that in most lipid classes, the major lipid species (Table 4.3) were mainly esterified to fatty acids with 16 carbons or 18 carbons and 2 double bonds, *e.g.* PC 36:4 (PC 18:2_18:2) or MGDG 34:4 (MGDG 16:2_18:2). These results are supported by GC-MS analysis, in which the most abundant FAs found in C-Honey and C-White were C18:2 (*n*-6), C16:0 and C18:1 (*n*-9). Lipid species with higher abundance per class were the same for C-White and C-Honey, except in DGTS and CL classes. The most abundant lipid species of C-Honey and C-White were the same as those previously identified in the *C-Hetero* strain but not in the wild-type C-Auto strain [9]. These differences between the lipid profile of the wild-type and the mutant strains may be due to the heterotrophic growth conditions. Heterotrophy, in *C. vulgaris*, induces a decrease in lipid species esterified with ALA and an increase in lipid species esterified with LA [9]. Some studies have also reported that mutagenesis can lead to pleiotropic effects in microalgae, affecting several untargeted genes, namely genes that encode enzymes involved in lipid metabolism [41]. In particular, it has been reported that the use of norflurazon can inhibit fatty acid Δ6 desaturases involved in the PUFA pathway [53,54]. Our results suggest that norflurazon can also affect the *ω3* desaturase, since C-White, which was the most exposed to this compound, was the microalgae with lower content of ALA and polar lipids esterified with ALA.

The most abundant phospholipid species were PC 36:4, PE 34:2, PG 34:1 and PI 34:2. For the LPC and LPE lipid classes, the most abundant species were LPC 18:2 and LPE 18:2. In C-White and C-Honey, DGDG 34:1, MGDG 34:4, SQDG 32:0 and DGGA 34:1 were the most abundant glycolipids. Among these, it is important to highlight SQDG 32:0, which has already been reported in C-Auto and C-Hetero [9] and is known to have anti-tumour activity [55].

Table 4.3. The number of lipid species identified by lipid class in C-Honey and C-White lipid extracts and with identification of the main lipid species by class.

Lipid classes	**Number of lipid species**		**Major lipid species**	
	C-White	**C-Honey**	**C-White**	**C-Honey**
Glycolipids	54	54		
DGDG	28	28	DGDG 34:1|DGDG 16:0_18:1	DGDG 34:1|DGDG 16:0_18:1
MGDG	21	21	MGDG 34:4|MGDG 16:2_18:2	MGDG 34:4|MGDG 16:2_18:2

SQDG	4	4	SQDG 32:0	SQDG 32:0
DGGA	1	1	DGGA 34:1|DGGA 16:0_18:1	DGGA 34:1|DGGA 16:0_18:1
Phospholipids	131	131		
PC	36	36	PC 36:4|PC 18:2_18:2	PC 36:4|PC 18:2_18:2
PE	42	42	PE 34:2|PE 16:0_18:2	PE 34:2|PE 16:0_18:2
PG	23	23	PG 34:1|PG 16:0_18:1	PG 34:1|PG 16:0_18:1
PI	20	20	PI 34:2|PI 16:0_18:2	PI 34:2|PI 16:0_18:2
PS	1	1	PS 34:2|PS 16:0_18:2	PS 34:2|PS 16:0_18:2
CL	2	2	CL 72:9|CL 18:2_18:2_18:2_18:3	CL 72:11|CL 18:2_18:3_18:3_18:3
LPC	6	6	LPC 18:2	LPC 18:2
LPE	1	1	LPE 18:2	LPE 18:2
Betaine lipids	4	8		
DGTS	4	8	DGTS 40:10|DGTS 20:5_20:5	DGTS 32:3|DGTS 16:0_16:3
Sphingolipids	18	18		
Cer	18	18	Cer 43:0;4O|Cer 19:0;3O/24:0;(2OH)	Cer 43:0;4O|Cer 19:0;3O/24:0;(2OH)
Total	207	211		

Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; DGGA, diacylglycerylglucuronide; SQDG, sulfoquinovosyldiacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserines; CL, cardiolipins; Cer, ceramide.

After the relative quantification of the data, a statistical analysis was carried out by comparing the profile of the two strains. Principal component analysis (PCA) of log-transformed levels of all individual lipid species identified (**Figure 4.1**) showed discrimination between the two strains. The first two principal components accounted for 96.9% of the total variance in the dataset (PC1 (91.2%) and PC2 (5.7%)), suggesting a clear difference in the RA of some groups of lipids in C-Honey and C-White. The lipid species that contributed to the major discrimination in PC1 were PE species, but also DGTS, PC, DGDG, and PG as can be observed in the boxplots in **Figure 4.1** 81 % of lipids were structural lipid species, mainly present in membranes (DGTS, PC and PE) and only 19 % were constituents of chloroplastic membranes (DGDG and PG).

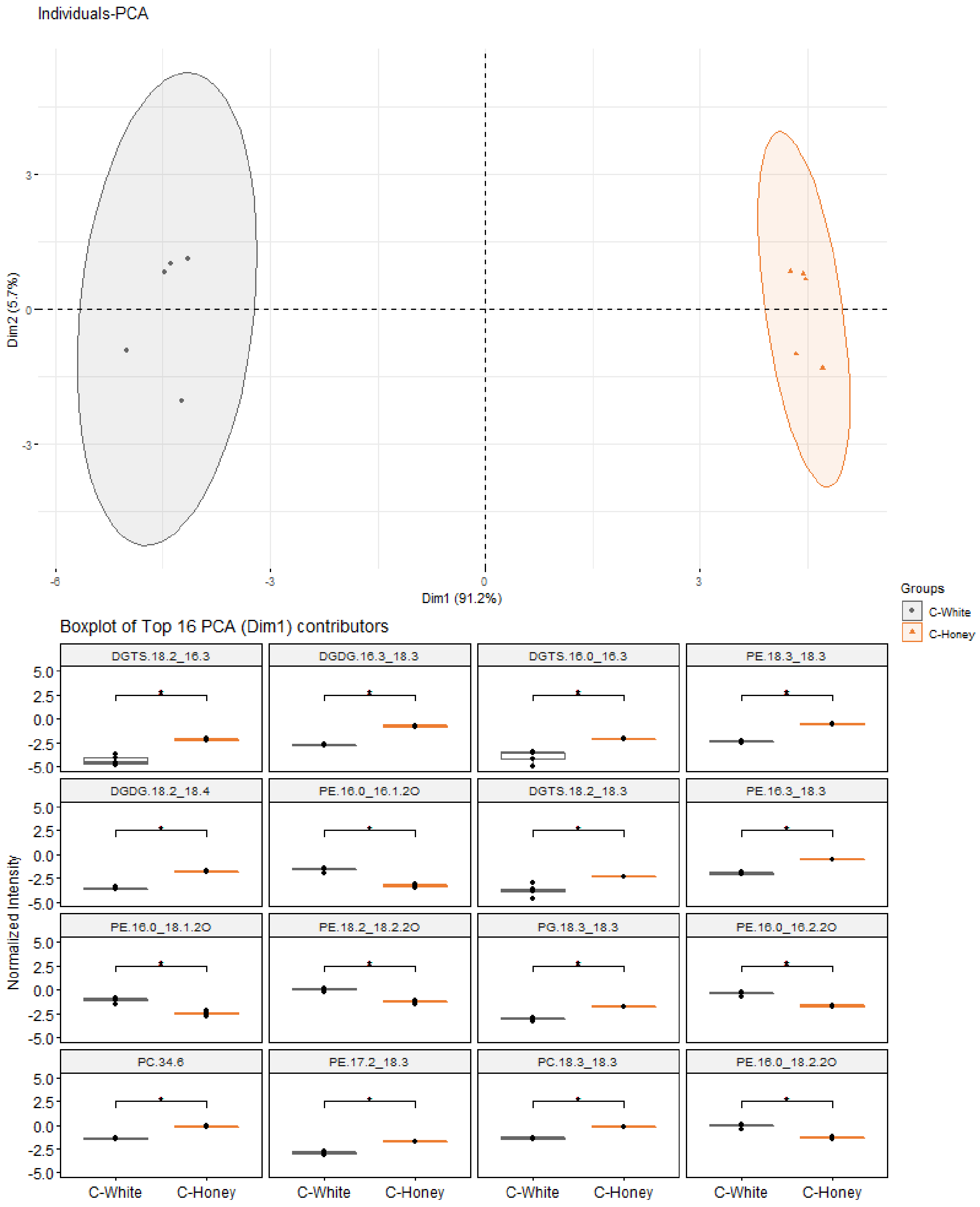

Figure 4.1. (**A**) Principal component analysis score plot of C-Honey (orange colour) and C-White (grey colour) *C. vulgaris*, based on LC-MS/MS. (**B**) Box plots of the 16 main contributors to PC1 discrimination. Abbreviations: DGDG, digalactosyldiacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol. *Statistically significant differences between strains of *C. vulgaris* (*Wilcoxon rank sum test*, $q < 0.05$).

Univariate analysis revealed a total of 169 significantly different lipids (80%) of the total pool of polar lipid species between the new *C. vulgaris* strains, supporting a strain-specific polar lipid profile (**Supplementary Table S4.5**). Therefore, to visualize lipid variations between algae, a two-dimensional hierarchical cluster analysis (HCA) was also applied to our dataset (**Figure 4.2**). The HCA includes the 50 most significant different lipid species (*Welch's t-test, p* < 0.05) in our study and reveals clear discrimination between C-Honey and C-White samples. The results show that the lipid species are clustered into two groups, a first cluster comprising 7 lipid species of three classes, PE, PC and MGDG, strongly expressed in C-White, and a second group comprising 43 lipids species highly expressed in C-Honey (Cer, PE, PC, PG and DGDG).

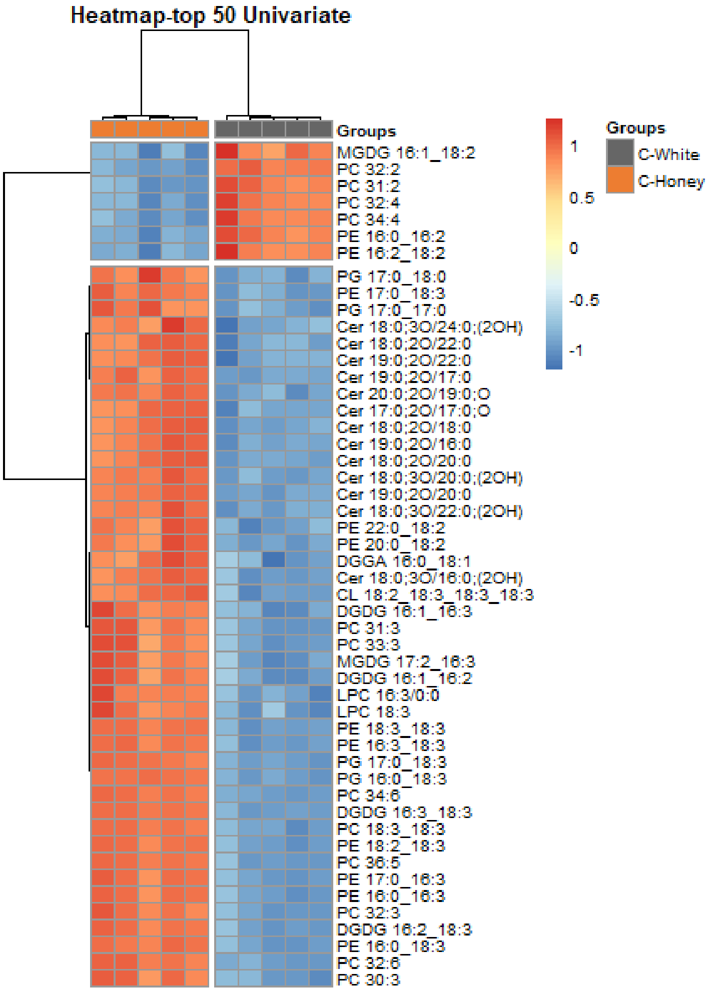

Figure 4.2. Two-dimensional hierarchical cluster analysis of LC-MS lipid profile. This dataset contains the 50 most significant polar lipids between strains of *C. vulgaris* (Welch's t-test, $p < 0.05$). Relative abundance levels are indicated on the colour scale, with numbers indicating the fold difference from the mean. The clustering of sample groups is represented by the dendrogram at the top, showing two main clusters, one for each group. The clustering of individual lipid species considering their similarity in relative abundance is represented by the

dendrogram on the left. Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; DGGA, diacylglycerylglucuronide; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipins; Cer, ceramide.

Few glycolipid species showed significant variation between strains. The glycolipid classes DGDG and MGDG are the main components of plastid lipids and chloroplasts, playing a role in the photosynthesis and signalling processes in cells. We identified in the two strains of *C. vulgaris* two statistically different species of MGDG (32:2 and 34:1) and one not significantly different changed MGDG (32:0), which have been reported in the literature with biological activities namely, anti-viral, anti-obesity and anti-tumour [56–58]. Glycolipids are also known to have several bioactive properties (such as anti-inflammatory), thus making microalgae extracts interesting for the nutraceutical and functional food or pharmaceutical industry.

Additionally, among the significantly different classes between the two strains evaluated, shown in the boxplots in Figure 4.3 and **Supplementary Table S4.6,** it is clear that C-White is richer in MGDG, while C-Honey is richer in Cer. The identified ceramide species were more abundant in C-Honey than in C-White, as can be seen in Figure 4.2 and **Supplementary Figure S4.2**. Several skin diseases have been associated with abnormal Cer content or composition, such as Gaucher disease, atopic dermatitis, and psoriasis, among others [59]. Ceramides are considered important lipid compounds for skin barrier function, being incorporated into skincare products to improve or restore skin barrier function [59]. A previous review also discussed the role of algal lipids as modulators of inflammation in skin diseases, as several phospholipids and glycolipids may play an important role in reducing inflammatory processes [60]. This behavior has been described for several classes of lipids (MGDG, DGDG, DGTS, SQDG, PC and PG) also highlighted in this study.

Despite the content in DGTS species being higher in C-Honey samples, DGTS 40:10 (DGTS 20:5_20:5) had a higher abundance in C-White (**Supplementary Figure S4.6**). This lipid species is associated with anti-inflammatory activity due to reducing the levels of nitric oxide (NO) [61]. In microalgae, it has been described that omega-3 PUFAs, such as alpha-linolenic acid, C18:3(*n*-3), and eicosapentaenoic acid, C20:5(*n*-3), are mainly incorporated into more complex classes of lipids, such as betaine lipids (DGTS) and glycolipids (MGDG, DGDG, and SQDG) [62]. Thus, this suggests that lipids from these classes may have a higher anti-inflammatory effect, contributing to the health effects of these lipid extracts and their potential use in functional foods and nutraceutical formulations.

Altogether, our data suggest that C-Honey and C-White can be considered promising alternatives for the functional food industry, as well as the cosmetic and cosmeceutical industry, in the formulation of skincare products, but also for the pharmaceutical industry.

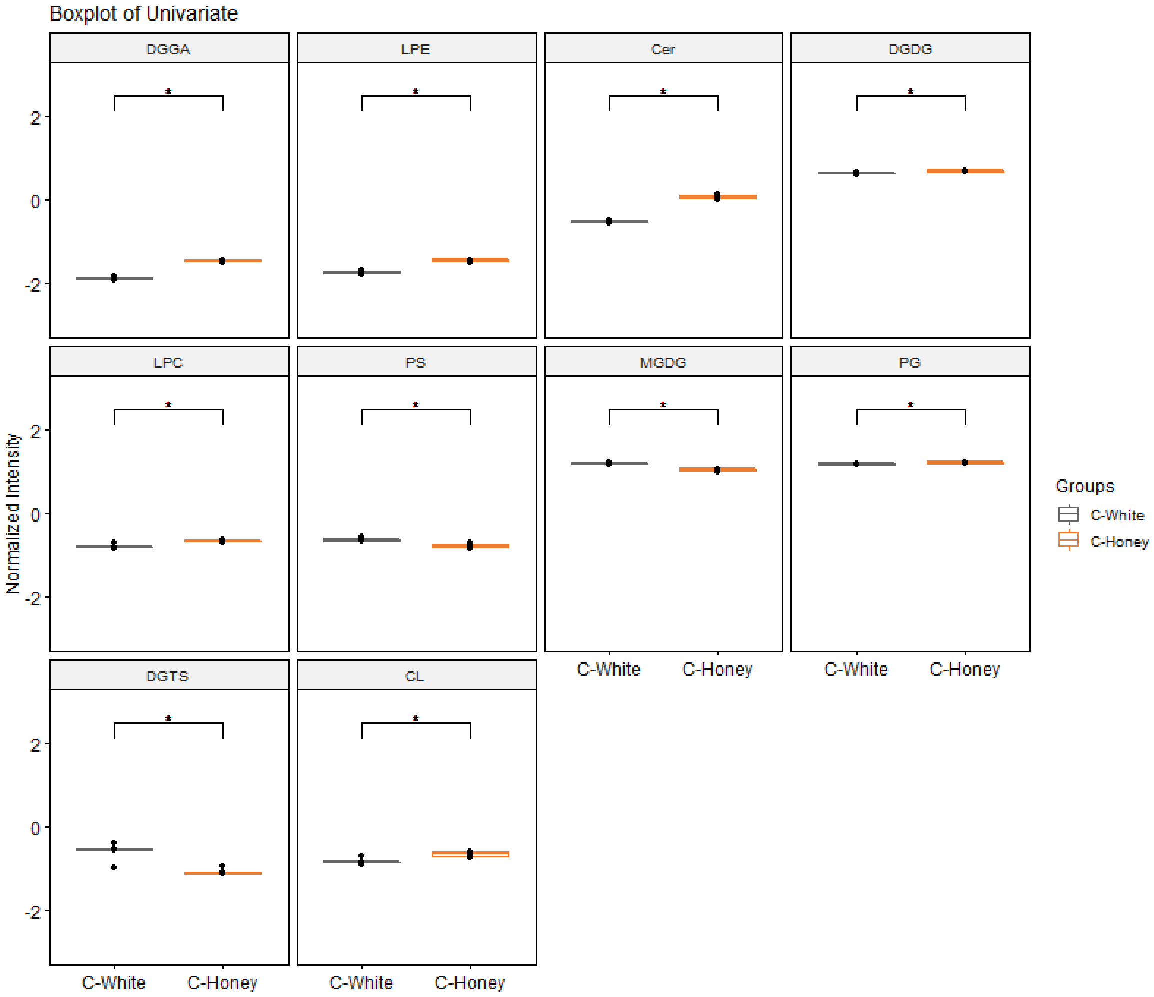

Figure 4.3. Box plots of significant lipid classes of C-White (grey colour) and C-Honey (orange colour) *C. vulgaris*. Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; DGGA, diacylglycerylglucuronide; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; CL, cardiolipin; Cer, ceramide.* Statistically significant differences between *C. vulgaris* strains (*Wilcoxon rank sum test*, $q < 0.05$).

3.4. Antioxidant activity of lipid extracts of C-Honey and C-White *C. vulgaris*

The antioxidant activity of C-Honey and C-White was measured for both lipid extracts. Lipid extracts of C-Honey had higher antioxidant activity in both scavenging assays (ABTS$^{\bullet+}$and DPPH$^{\bullet}$) compared to C-White, as shown in Figure 4.4. As the concentration of microalgae extracts evaluated did not promote 50% inhibition (IC50) of the radicals, IC10 was determined in both assays.

In the ABTS$^{\bullet+}$ assay, for a 10 % radical inhibition, 55.1 ± 4.7 μg mL^{-1} of the C-Honey lipid extract was required, representing a Trolox equivalent (TE) of 72.0 ± 6.6 μmol Trolox g^{-1} lipid extract. A higher concentration of C-White lipid extract (263.1 ± 50.1 μg mL^{-1}, with a TE of 15.6 ± 3.4 μmol Trolox g^{-1}) was required to obtain the same percentage inhibition. In the DPPH$^{\bullet}$ assay, the trend was similar, C-Honey showed a lower IC10 value (64.8 ± 4.1 μg mL^{-1}, with a TE of 77.3 ± 5.0 μmol Trolox g^{-1}) and C-White the higher value (324.1 ± 22.3 μg mL^{-1}, with a TE of 15.5 ± 1.0 μmol Trolox g^{-1}). These results showed that the two strains of *C. vulgaris* exhibited antioxidant potential but at different levels, with C-Honey exhibiting the highest antioxidant potential.

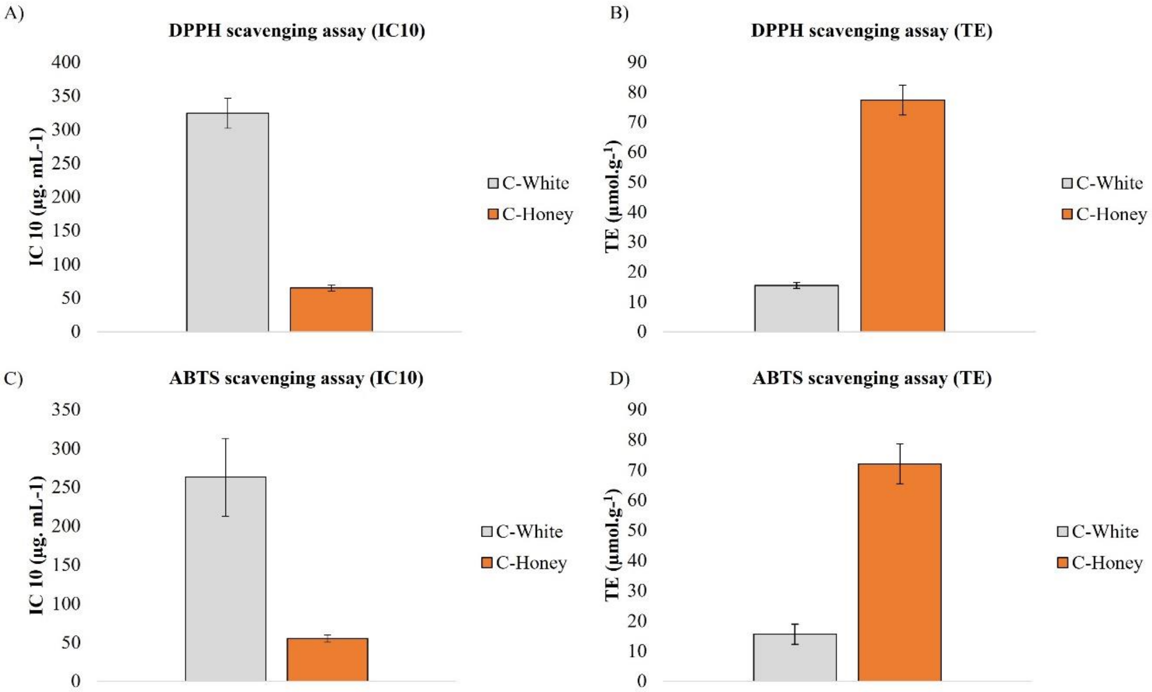

Figure 4.4. Antioxidant activity of C-Honey and C-White lipid extracts. The amount of lipid extract (μg mL^{-1}) required to induce 10 % inhibition: (**A**) DPPH$^{\bullet}$ radical (**B**) ABTS$^{\bullet+}$radical, and (**C,D**) Trolox equivalents (TE) (μmol Trolox g^{-1} of lipid extract). Data are means ± SD, n=3.

These new strains of C. *vulgaris* had lower antioxidant activity compared to previously reported values for C-Hetero and C-Auto [9]. In the literature, the two strains had similar antioxidant potential, at a concentration of 250 µg mL^{-1}, promoting 100% inhibition of the $ABTS^{\bullet+}$ radical and 64% and 59% inhibition of the $DPPH^{\bullet}$ radical for C-Hetero and C-Auto, respectively [9].

The antioxidant properties of lipid extracts have been correlated with the presence of PUFAs [34]. The lipid extracts of C-Honey and C-White, showed similar content in the total amount of PUFAs (44.1 % *vs* 41.9 %), but different antioxidant activities. Thus, the dissimilar antioxidant activity could be correlated with the *n*-6/*n*-3 ratio (2.1 *vs* 13.9) for C-Honey vs C- White, and low abundance of omega-3 PUFAs of C-White that showed lower antioxidant activity. However, differences between the antioxidant effect of omega-3 and omega-6 PUFAs are not yet fully understood and also some authors have suggested that omega-3 and omega-6 PUFAs are prone to oxidation to the same extent [34]. Nevertheless, the composition of the lipid extracts is quite complex, and the contribution of the synergistic effects of other compounds with antioxidant properties, such as some pigments, cannot be ruled out. For example, the ability of pigments to scavenge oxidative radicals is well established and for this reason, the abundance and the nature of the pigments present in lipid extracts will also impact their antioxidant potential. The chlorophyll contents between these new strains are quite different [6] from other *C. vulgaris* as they are deficient in chlorophyll, which also supports the lower antioxidant potential verified for these strains.

Regarding the application of these extracts as food ingredients with antioxidant properties, C-Honey was the most promising. This is mainly due to the fact that this strain is richer in omega-3 PUFAs and has higher antioxidant activity, in addition to certain organoleptic factors such as the attenuated colour, taste and odour preferred by consumers.

In addition to the antioxidant activities of the lipid extracts from *C. vulgaris,* other bioactivities with important effects on human health have also been described for these algae lipids, including antitumoral [63], antibacterial [64] and anti-inflammatory [9]. Among these, we also evaluated the anti-inflammatory potential of the lipid extracts of the two mutants of *C. vulgaris*.

3.5. Anti-inflammatory activity of lipid extracts of C-Honey and C-White C. vulgaris

The anti-inflammatory potential of lipid extracts from C-Honey and C-White, was determined by measuring inhibition of COX-2 activity for three concentrations (20, 60, 125 μg mL^{-1}). It was observed that only the most concentrated lipid extracts (125 μg mL^{-1}) exhibited potential anti-inflammatory activity (**Table 4.4**). At this concentration, C-White extracts showed higher anti-inflammatory activity (34.9 ± 1.5 %) than C-Honey extracts (13.6 ± 10.5 %).

Table 4.4. Anti-inflammatory potential (COX-2 assay) from lipid extracts (125 μg mL^{-1}) of C-Honey and C-White. The results were expressed as a percentage of COX-2 inhibited.

	COX-2 activity (%)	
Concentration of lipid extract (μg mL^{-1})	C-White (%)	C-Honey (%)
125	34.9 ± 1.5	13.6 ± 10.5

These new strains showed lower anti-inflammatory activity compared to the lipid extracts of C-Hetero (86.1 ± 0.5 %) and C-Auto (86.5 ± 4.5 %) reported in the literature [9]. However, in this paper, the microalgae were tested at a higher concentration (500 μg mL^{-1}), which could explain the higher anti-inflammatory activity observed for C-Auto and C-Hetero compared to the strains evaluated in the present study, although it has been described that anti-inflammatory activity does not exhibit a dose-dependent response [65,66]. Moreover, compared to other microalgae tested at similar concentrations, the new strains had lower COX-2 inhibitory potency than *Chlorococcum amblystomatis* [65], while C-White showed similar activity to cyanobacteria *Gloeothece* sp. [66].

Interestingly, C-White showed higher anti-inflammatory activity than C-Honey, which can be explained by the higher abundance of LA and of MGDG and oxidized PE species, reported in the FA and lipidomic analysis, respectively. Termer and co-workers reported an inhibitory effect of LA against COX-2 [67]. MGDG is reported with anti-inflammatory properties [68]. Recently, there is growing interest in the role of oxidized phospholipids (OxPLs), since these compounds mediate inflammatory processes, such as the induction and resolution of inflammation [69]. OxPLs can indeed have a pro or anti-inflammatory role, and, therefore, may contribute to the anti-inflammatory activity of C-White extracts. This is still an area that has not yet been fully explored and which requires additional studies to assess the potential, functional or pharmacological effect of C-White.

4. Conclusions

This work described the differences in FA and polar lipid profile, as well as the antioxidant and anti-inflammatory properties of two *C. vulgaris* (C-Honey and C-White). These two mutant strains (obtained by chemically induced random mutagenesis from *C. vulgaris* in heterotrophic culture) are already approved for human consumption and marketed as food ingredients and supplements. The same lipid classes as well as the same major lipid species (by lipid class) were found in the polar lipidome of C-Honey and C-White, and consistent with what had been previously reported for the reference strain produced in heterotrophic conditions. Despite the similarities, statistical analysis showed a clear difference in the relative abundance of certain lipids species and classes, revealing a strain-specific polar lipid signature. C-Honey was richer in PE and DGDG species esterified with omega-3 polyunsaturated fatty acids and Cer species, while C-White was richer in lipids esterified with saturated fatty acids and omega-6 polyunsaturated fatty acids, as well as MGDG and oxidized PEs. The lipid signature of each new strain may explain the observed differences in its anti-inflammatory and antioxidant activities. C-Honey showed the best antioxidant potential and C-White the best anti-inflammatory activity. However, further studies are needed to understand the mechanisms of anti-inflammatory activity of putative bioactive lipids, especially using cell lines such monocytes or macrophages and in animal models. Furthermore, in the future, transcriptomic analyses will also provide insight into the biological mechanisms of lipid metabolism in *C. vulgaris* grown under different culture conditions. These will contribute to the added value of these strains as food supplements and nutraceuticals. The polar lipid composition of C-Honey and C-White and their biological activities, described in this study, as well as their improved organoleptic properties and high protein content, contribute to the valorization of the two strains not only as new ingredients and food supplements with nutritional value but also as a promising alternative for the nutraceutical, cosmeceutical and pharmaceutical industries.

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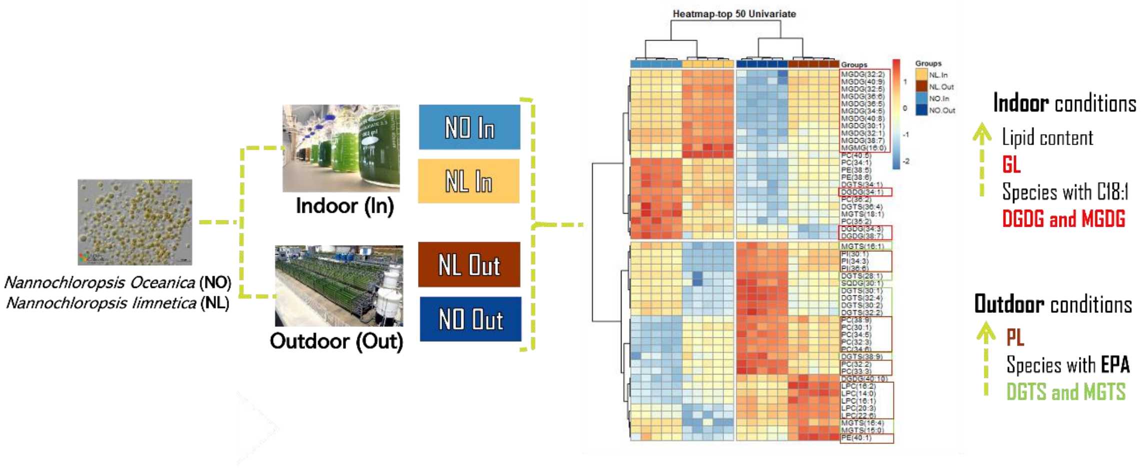
CHAPTER 5 - Effects of outdoor and indoor cultivation on the polar lipid composition and antioxidant activity of *Nannochloropsis oceanica* and *Nannochloropsis limnetica*: a lipidomics perspective



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Effects of outdoor and indoor cultivation on the polar lipid composition and antioxidant activity of *Nannochloropsis oceanica* and *Nannochloropsis limnetica*: a lipidomics perspective

Abstract: *Nannochloropsis* is a genus of eicosapentaenoic acid-rich microalgae with high levels of value-added polar lipids. However, the polar lipid composition of microalgal biomass is highly dependent on culture conditions (*e.g.*, light or temperature), which are significantly different under indoor and outdoor culture conditions. In this study, we sought to investigate the plasticity of the polar lipid profile of a marine (*N. oceanica*) and a freshwater (*N. limnetica*) species of *Nannochloropsis* grown in indoor and outdoor photobioreactors. To this end, the polar lipidome and fatty acid profiles were characterized by liquid chromatography-mass spectrometry (LC-MS), and gas chromatography-mass spectrometry (GC-MS), respectively. In addition, the antioxidant activity of their lipid extracts was assessed. The highest lipid contents were obtained for the two species grown indoors. LC-MS analysis identified 239 different polar lipid species, of which 220 were shared by all experimental groups. Candidate lipid biomarkers from both culture systems were proposed, including MGDG(34:2), MGDG(34:1) and PG(36:6). For both species, indoor conditions lead to lipid extracts rich in glycolipids and higher in oleic acid content. In contrast, outdoor conditions lead to higher proportions of phospholipids and betaine lipids and a higher relative content of eicosapentaenoic acid (EPA). The polar lipid profile of the two *Nannochloropsis* species differed primarily in the relative amounts of certain betaine lipids, mainly DGTS (which was increased in *N. oceanica*) and lysolipids (LPC, and LPE) (increased in *N.limnetica*), although the majority of lipids were observed in both species. The lipid extracts showed antioxidant activity (IC_{15}) ranging from 30.4 ± 1.8 to 45.7 ± 1.6 µmol Trolox g^{-1} of lipid extract. Overall, this study provides insight into the lipid metabolic adaptation of two *Nannochloropsis* species, providing the know-how to obtain a healthy polar lipid-rich biomass useful for novel applications in pharmaceutical, nutraceutical, or novel foods.

Keywords: Microalgae, *Nannochloropsis oceanica*, *Nannochloropsis limnetica*, Lipidomics, Growth conditions, Polar lipids

1. Introduction

Nannochloropsis is a genus of microalgae that belongs to the class Eustigmatophyceae, phylum Ochrophyta, traditionally comprising six known species, *N. gaditana*, *N. salina*, *N. oculata*, *N. granulata*, *N. oceanica,* and *N. limnetica* [1]. However, Fawley et al proposed a separate genus (*Microchloropsis*) comprising the species *M. gaditana* and *M. salina* (originally classified as *N. gaditana* and *N. salina*) and described a new species, *N. australis* [2]. These microalgae are found in marine and freshwater habitats and are characterized by high robustness that allows their cultivation on an industrial scale in open ponds and photobioreactors [3,4]. Nevertheless, production in closed photobioreactors with controlled biotic and abiotic conditions provides more reproducible biomass with a constant biochemical composition, crucial for commercialization and other applications (*e.g.*, livestock feed and poultry, aquaculture) [4,5]. Currently, the biomass of *Nannochloropsis* sp. is not approved for human consumption [4]; however, its extracts enriched with bioactive compounds are currently commercialized as food supplements in the USA [6,7], in different applications such as cosmeceutical [8], pharmaceutical [8], and nutraceutical [9]. *Nannochloropsis* spp. feed ingredients have been shown to improve growth performance and immune response in marine animals [10], and extracts of *N. gaditana*, have shown *in vitro* dermo protective effects against oxidative stress [11]. The reported benefits of *Nannochloropsis* sp. are mainly associated with their bioactive composition of key molecules such as *n*-3 polyunsaturated fatty acids (PUFAs) [9].

The biomass of *Nannochloropsis* sp. is widely used as a source of healthy and essential lipids due to its high content of these metabolites, ranging from 6 to 60 % of dry weight [4,12]. Among these lipids, *Nannochloropsis* species are known to be rich in *n*-3 PUFAs, mainly eicosapentaenoic acid (EPA, C20:5 *n*3), with contents ranging from 1.1 % to 12 % of its dry weight [3]. Therefore, these microalgae are considered a sustainable and promising alternative to fish oil [13]. EPA is an essential constituent of the membranes of the central nervous system, where it plays unique and beneficial roles. Its dietary intake is therefore crucial for humans and other animals. The Food and Agriculture Organization [14] and the European Food Safety Authority [15] have recommended a minimum daily intake of 250 mg of EPA + docosahexaenoic acid (DHA) per adult. Adequate intake of EPA is essential for supporting heart health, preventing cardiovascular disease, reducing inflammation, and lowering triglyceride (TG) levels in patients with hypertriglyceridemia [16].

Recently, it has been reported that the benefits of *n*-3 PUFAs depend on their free form and are also related to the lipids that carry them. Previous studies have reported that *n*-3 PUFAs esterified to complex polar lipids, such as phospholipids (PLs) and glycolipids (GLs), especially of marine origin, are more bioavailable than those esterified to neutral lipids [17]. PLs and GLs, are more abundant in the biomass of microalgae than free fatty acids (FFAs), as shown on *Nannochloropsis* sp. [18]. The beneficial health properties of *Nannochloropsis* sp. polar lipids have well-known bioactivities, including anti-inflammatory [19] and anti-atherogenic [20]. In addition, recent clinical studies have shown that supplementation with an EPA polar lipid extract of *Nannochloropsis* sp. increased the *n*-3 index and EPA concentration and decreased the total cholesterol levels in healthy individuals [21]. Furthermore, the consumption of oil rich in polar lipids from *N. oculata* promoted a higher concentration of EPA in human plasma than krill oil [22]. The biological relevance of these molecules led to the characterization of the polar lipid signature of four species of the genus *Nannochloropsis*, namely *N. gaditana* [23], *N. salina* [24,25], *N. oculata* [26–28], and *N. oceanica* by liquid chromatography-mass spectrometry (LC-MS) [29,30].

Different growth phases, growth conditions, and environmental stimuli induce a change in the polar lipid composition of microalgae of the genus *Nannochloropsis* [25,27,30–32]. For example, in *N. oceanica*, nitrogen deprivation and exposure to intense light induced a decrease in the amount of monogalactosyldiacylglycerol (MGDG), phosphatidylglycerol (PG), diacylglyceryl-O-4′-(N, N, N,-trimethyl) homoserine (DGTS) and phosphatidylcholine (PC) and an accumulation of digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) [30]. However, changes in the polar lipid composition of *Nannochloropsis* spp. due to the manipulation of the growing conditions remains scarcely addressed, hampering the optimization of biomass production to obtain high quality and high-value *Nannochloropsis* biomass in an industrial setting. Thus, in this work, we characterized, for the first time, the polar lipidome of freshwater *N. limnetica*, and evaluated the lipidome plasticity of this alga as well as of marine *N. oceanica* cultivated outdoors and indoors. To this end, we identified and quantified the FAs and the polar lipids from the extracts of *N. limnetica*, and *N. oceanica* and determined the antioxidant capacity of resulting lipid extracts.

2. Materials and methods

2.1. Microalgae strain and culture media

Nannochloropsis oceanica 0011NN and *Nannochloropsis limnetica* 0065NA were cultivated under autotrophic conditions in the production facility of Allmicroalgae (Leiria, Portugal). The composition of Guillard's F/2 culture medium was adapted to the local water, using sodium nitrate adjusted to 10 mM as the nitrogen source. All species were supplemented with 25 µM of iron and a supplement enriched in magnesium (Necton, Olhão, Portugal), the saline species was supplemented with commercial sodium chloride (Necton, Olhão, Portugal) to a final salinity of 30 g L^{-1}.

2.2. Laboratory-scale cultivation

The experiments were carried out in 1-L flask reactors until the stationary growth phase was reached. The cultures were maintained with constant aeration (0.2-µm-filtered atmospheric air), under a continuous photon flux density of 100 µmol photons $m^{-2}.s^{-1}$, at 24 °C. The pH of the cultures was maintained between 7.0 and 8.0 by periodic injection of CO_2. Samples were taken every other day for assessment of culture growth and microbiological monitoring.

2.3. Flat-Panel cultivation

Microalgae cultures were expanded from 5-L flask reactors grown in the laboratory under the conditions mentioned above. Four 5-L glass reactors of each strain were used as pre-inoculum for 75-L Flat Panels (FP), which were used to inoculate the FPs used in the assay. The assay took place from January 7 to January 27, 2020. The assay started at 0.3 g $L^{-1,}$ and cultures were followed until the stationary phase was reached. The pH was maintained between 7 and 8 by pulsed injections of CO_2 and continuous aeration. During this time, the maximum temperature and radiation were below 18 °C and 500 w m^{-2}, with the lowest temperature and radiation recorded at 0 °C and 0 w m^{-2}, respectively (**Supplementary Figure S1**).

2.4. Reagents

HPLC grade dichloromethane (CH_2Cl_2), absolute ethanol 96 % (CH_3CH_2OH), and methanol (CH_3OH) were purchased from Fisher Scientific Ltd. (Loughborough, UK). The water was of Milli-Q purity (Synergy1, Millipore Corporation, Billerica, MA, USA). $DPPH^{\bullet}$ was purchased from Aldrich (Milwaukee, WI, USA). All other reagents were purchased from major commercial sources. Lipid internal standards were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA): 1,2-dimyristoyl-*sn*-glycero-3-phospho-(10-rac-glycerol) (dMPG), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (dMPC), 1-nonadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (dMPE), N-heptadecanoyl-D-erythro-sphingosine (Cer(17:0/d18:1)).

2.5. Extraction of total lipids

Lipid extraction was performed as previously described [33]. Briefly, 25 mg of spray-dried biomass was extracted four times using 2 mL of CH_2Cl_2:CH_3OH (2:1, v/v). The resulting supernatants were combined and dried under a stream of nitrogen. The dried extracts were then re-extracted with 2 mL CH_2Cl_2, 1 mL CH_3OH and 0.75 mL Milli-Q water, and the resulting organic layers were collected. The aqueous phase was re-extracted twice with 2 mL CH_2Cl_2. The combined organic phases were dried under a stream of nitrogen and labelled as lipid extracts. The lipid content was calculated as a percentage of dry weight, using Eq. (1):

$$Lipid\ content\ \left(\%DW, \frac{w}{w}\right) = \frac{Weight\ of\ lipid\ extract\ (mg)}{Weight\ of\ biomass\ (mg)} x100\ (1)$$

Five technical replicates were performed for each group of samples.

2.6. Antioxidant activity

The antioxidant scavenging activity against the α,α-diphenyl-β-picrylhydrazyl radical ($DPPH^{\bullet}$) was evaluated using a method previously described [34] with some modifications detailed in [33]. Briefly, 150 µL of an ethanolic dilution of the lipid extracts (25, 125, 250, 500 µg mL^{-1}) were mixed with 150 µL of a $DPPH^{\bullet}$ working solution in ethanol (absorbance ~0.9). Absorbance was measured at 517 using a UV-vis spectrophotometer (Multiskan GO

1.00.38, Thermo Scientific, Hudson, NH, USA). All measurements were performed in triplicate. The antioxidant activity, expressed as percentage inhibition of the DPPH$^{\bullet}$ radical, was calculated using Eq. (2):

$$\text{Inhibition \%} = \frac{(\text{AbsDPPH} - (\text{AbsSample} - \text{AbsControl}))}{\text{AbsDPPH}} \text{x}100 \ (2)$$

The activity expressed in Trolox Equivalents (TE) was calculated using Eq. (3), where the IC15 values are the concentration of the sample or of Trolox, which induces the reduction of the DPPH$^{\bullet}$ radical in 15 %:

$$\text{TE } (\mu\text{mol/g}) = \frac{\text{IC 15 Trolox } (\mu\text{mol/g})}{\text{IC 15 of samples } (\mu\text{g/mL})} \text{x}1000 \ (3)$$

2.7. Fatty Acid Analysis

Fatty acid methyl esters (FAMEs) were prepared from lipid extracts (equivalent to 15 µg of phospholipid) as previously described [35]. A solution of methyl nonadecanoate (internal standard) at 10.0 µg mL^{-1} was added to the FAMEs solution (Sigma, St. Louis, MO, USA). Gas chromatography-mass spectrometry (GC–MS) analyses were performed on an Agilent Technologies 8860 GC System (Santa Clara, CA, USA) interfaced with an Agilent 5977B Mass Selective Detector (Agilent, Santa Clara, CA, USA) with an electron impact ionization (70 eV) and scanning the range of m/z 50–550 in a 1 s cycle using full scan mode acquisition. A DB-FFAP capillary column (30 m long x 0.32 mm internal diameter, 0.25 µm film thickness (J&W Scientific, Folsom, CA, USA)) was used. The following conditions were used during chromatographic analysis: injection volume 2 µL (splitless), a constant flow rate of 1.4 mL min^{-1} of helium gas, inlet detector temperature 230 °C, temperature 220 °C. The oven temperature was programmed as follows: 58 °C for 2 min, 25 °C min^{-1} to 160 °C, 2 °C min^{-1} to 210 °C, 30 °C min^{-1} to 225 °C (held for 10 minutes). The data acquisition software used was GCMS5977B/Enhanced MassHunter. The Agilent MassHunter Qualitative Analysis 10.0 software was used to identify FAs. The identification of FAs was carried out by comparing the MS spectrum with the NIST library and confirmed by the literature. The relative abundance (RA) of fatty acids was calculated by the area percentage method, as previously described [33,36,37]. The content of fatty acids expressed in mg FA/100 mg of lipid extracts were obtained from six-point calibration curves of each FAME from a commercial FAME mixture (Supelco 37 Component FAME Mix, CRM47885, Sigma Aldrich, St. Louis, MO, USA), as previously detailed [38].

The atherogenic (AI), thrombogenic (TI) and hypocholesterolemic/ hypercholesterolemic indexes (h/H) were calculated according to the following formula (Eqs. 4, 5 and 6) [39,40].

$$\mathrm{AI}=\frac{[\mathrm{C12{:}0}+(4*\mathrm{C14{:}0})+\mathrm{C16{:}0}]}{[\sum\mathrm{MUFA}+\sum(n\text{-}6)+\sum(n\text{-}3)]} \tag{4}$$

$$\mathrm{TI}=\frac{[\mathrm{C14{:}0}+\mathrm{C16{:}0}+\mathrm{C18{:}0}]}{[(0.5*\sum\mathrm{MUFA})+(0.5*\sum(n\text{-}6))+(3*\sum(n\text{-}3))+(\frac{\sum(n\text{-}3)}{\sum(n\text{-}6)})]} \tag{5}$$

$$(\mathrm{h/H})=\frac{[\text{cis-C18:1}+\sum\mathrm{PUFA}]}{[\mathrm{C12{:}0}+\mathrm{C14{:}0}+\mathrm{C16{:}0}]} \tag{6}$$

2.8. Quantification of phospholipids

The phospholipid (PL) content in the lipid extracts was determined as detailed in [33]. Phosphate standards were prepared from a solution of $NaH_2PO_4.2H_2O$ with 100 μg/ml of P. Samples and standards were re-suspended in 125 μL of 70 % perchloric acid. The samples were heated for 1 hour at 180 °C in a heating block (Stuart, Staffordshire, UK). Then, 825 μL of milli-Q water, 125 μL of 2.5 % ammonium molybdate solution and 125 μL of 10 % ascorbic acid were added. Samples and standards were vortexed, incubated for 10 minutes at 100 °C in a water bath, and cooled to room temperature. The absorbance was measured at 797 nm in an ultraviolet-visible (UV–Vis) spectrophotometer (Multiskan GO, Thermo Scientific, Hudson, NH, USA). The amount of PL was calculated by multiplying the quantity of determined phosphorus (μg) by 25. Two duplicates of two independent measurements were carried out for each sample.

2.9. Quantification of glycolipids

The glycolipid (GL) content of the lipid extracts was determined as detailed in [33]. The glycolipid content was obtained by calculating the hexose content. The amount of sugar was read from a standard curve prepared by carrying out the reaction on known amounts of glucose. The absorbance was measured at 505 nm in a UV–Vis spectrophotometer

(Multiskan GO, Thermo Scientific, Hudson, NH, USA). Two duplicates of two independent measurements were performed for each sample.

2.10. Hydrophilic interaction liquid chromatography-electrospray ionization-mass spectrometry (HILIC–ESI–MS)

The lipid extracts were analyzed by hydrophilic interaction liquid chromatography (HILIC) in a Dionex Ultimate 3000 (Thermo Fisher Scientific, Bremen, Germany) using an Ascentis® Express column (10 cm x 2.1 mm, 2.7 µm; Sigma-Aldrich) coupled to the Q-Exactive® hybrid quadrupole Orbitrap mass spectrometer (Thermo Fisher, Scientific, Bremen, Germany). Mobile phase A consisted of water, acetonitrile, and methanol (25/50/25 %), with 5 mM ammonium acetate and mobile phase B was a mixture of acetonitrile and methanol (60/40 %) with 5 mM ammonium acetate. The following gradient was applied: 5 % A (0-2 min), followed by a linear increase to 48 % of mobile phase A in 8 min, and a further linear increase to 65 % A in 5 min, followed by a 2 minutes maintenance period and return to initial conditions within 3 min. Following elution, the column was re-equilibrated for 10 minutes.

To perform HILIC-MS analyses, a volume of 10 µL of each sample (equivalent to 10 µg of phospholipid), 82 µL of eluent (95 % of mobile phase B and 5 % of mobile phase A), and 8 µL of lipid standards mixture (dMPC - 0.04 µg, dMPE - 0.04 µg, LPC - 0.04 µg, dMPG - 0.024 µg, Cer(17:0/d18:1) - 0.04 µg), were mixed and 5 µL of this mixture was injected into the Ascentis® Express microbore column (10 cm x 2.1 mm, 2.7 µm; Sigma-Aldrich) at 35 °C and a flow-rate of 200 µL min^{-1}. The mass spectrometer operated simultaneously in positive (ESI 3.0 kV) and negative (ESI −2.7 kV) modes, as previously reported [41], except for the sheath gas flow rate, which was 20 U. MS/MS determinations were performed as described in [41] but with a normalized collision energy™ (CE) ranged between 25, 30, and 35 eV.

For lipidomic analysis, the identification of molecular species was based on the *m/z* ratio of the ions observed in LC-MS spectra, mass accuracy (< 5 ppm), LC retention time, and manual interpretation of LC-MS/MS spectra based on previous publications [33,42,43]. Integration of the ion peak area was performed using the MZmine software package (version 2.53) and included baseline correction, peak deconvolution, deisotoping and alignment, and gap-filling [44]. Peaks of raw intensity less than 1 × 10^4 were excluded. Quantitation of lipid species was performed by dividing the integrated peak areas of each molecular ion by the peak area of the selected internal standards. Normalized peak areas of phospholipids or glycolipids species were subsequently divided by the injected amount of phospholipids or glycolipids, measured using colourimetric tests, as described previously [33]. The amount of each polar lipid class was estimated by the sum of all lipid species belonging to the lipid class. All the lipid species and lipid classes semi-quantified were log-transformed. The Venn diagram of all identified polar lipid species was performed using the open-source jvenn [45].

2.11. Statistical Analysis

Multivariate and univariate analyses were performed using R version 4.0.2 [46] in Rstudio version 1.3.1093 [47]. One-way ANOVA followed by Tukey's multiple comparison test was performed to compare lipid, phospholipid and glycolipid content and antioxidant activity. Whenever the normality assumptions were not verified, the Kruskal-Wallis test was employed. Kruskal-Wallis test followed by Dunn's post-hoc comparisons were performed to compare the profile of fatty acids, polar lipidome, and lipid classes. All data were log-transformed using the metaboanalyst software [48]. PCA and ellipses were created using R libraries FactoMineR [49] and factoextra [50]. Kruskal-Wallis test followed by Dunn's post-hoc comparisons were performed using the R package rstatix [51]. *P*-values were corrected for multiple testing using the BH Benjamini, Hochberg, and Yekutieli method (*q* values) [52]. A *q*-value < 0.05 was considered an indicator of statistical significance. Heatmaps and Hierarchical Cluster Analysis were created using the R package pheatmap using "Euclidean" as clustering distance and "ward.D" as the clustering method [53]. Heatmaps were constructed based on the 16 fatty acids or 50 polar lipid species with the lowest *q*-values in Dunn's Test. Heatmaps that were constructed for comparisons between growth conditions (Out versus In) by selecting the species with lower Dunn *q*-values between the relevant groups (NL In/NL out, NO In/NO out, NL In/NO out, NL out/NO in) and (NL Out/NO Out, NL In/NO In). Boxplots were created using the R package ggplot2 [54]. Finally, volcano plots for pairwise comparison using Welch's t-test were performed using the R library EnhancedVolcano [55].

3. Results

3.1. Lipid, phospholipid, and glycolipid content of *N. oceanica* and *N. limnetica* grown outdoors and indoors

The total lipid content (mg/100 mg of biomass dry weight (DW)), and the amount of phospholipids (PL), glycolipids (GL) and neutral lipids plus pigments (µg/mg of lipid extract DW) in lipid extracts were determined for *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In) (**Table 5.1)**.

The lipid content of *Nannochloropsis* samples ranged from 23.6 ± 2.6 to 35.3 ± 0.8 % of biomass dry weight. *N. oceanica* grown indoors (NO-In) had a higher total lipid content (35.3 ± 0.8 %) than the same species grown outdoors (NO-Out) (26.1 ± 1.6 %) ($q < 0.01$). No statistically significant differences were observed for *N. limnetica* (25.9 ± 4.0 % NL-In versus 23.6±2.6 % NL-Out) (**Table 5.1, Supplementary Table S5.1-Table S5.2**), or between species grown outdoors (NL-Out versus NO-Out). However, significant differences were found between species grown indoors (NL-In versus NO-In) ($q < 0.01$), being higher in NO-In (**Supplementary Table S5.1-Table S5.2)**.

PL content ranged from 57.6 ± 8.0 (NL-In) to 104.8 ± 3.3 (NO-Out) µg/mg lipid extract. Higher amounts of PLs in lipid extracts were recorded in microalgae grown outdoors ($q<0.001$) (**Table 5.1**). No statistically significant difference was observed between species grown outdoors (NL-Out versus NO-Out). However, as mentioned for LC, significant differences were found between species grown indoors (NL-In versus NO-In) ($q < 0.01$). The amount of neutral lipids and pigments in the lipid extract, which was estimated by the difference between the amount of total lipids and the sum of PLs and GLs, was not significantly different between groups (**Supplementary Table S5.1)**. The GL content ranged from 233.2 ± 10.0 (NL-Out) to 274.8 ± 11.0 (NO-In) µg/mg lipid extract. A higher GL content was found in NO-In (274.8 µg/mg in NO-In versus 235.3 µg/mg in NO-Out, $q < 0.05$). GL content was similar between species (NL versus NO) when grown under the same conditions (**Supplementary Table S5.1-Table S5.2)**.

Table 5.1. Total lipid extract of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In) and the amount of phospholipids (PL), glycolipids (GL), and neutral lipids as well as pigments in the total lipid extracts and in the biomass. The corresponding lipid yield was expressed as a percentage, taking into account the weight of the dried biomass. The GL, PL and neutral lipids and pigments were expressed as µg per mg of dry weight lipid extract (DW) and as mg per 100 mg of biomass DW. Data are means ± SD, n=5.

Growth conditions	Total lipid extract (%) (mg/100 mg biomass DW)	PL (µg/mg lipid extract DW)	GL (µg/mg lipid extract DW)	Neutral lipids + pigments (µg/mg lipid extract DW)	PL (%) (mg/100 mg biomass DW)	GL (%) (mg/100 mg biomass DW)	Neutral lipids + pigments (%) (mg/100 mg biomass DW)
				Mean ± STD			
NO-Out	26.1±1.6	104.8±3.3	235.2± 12.5	660±12.5	2.7±0.2	6.1±0.2	17.2±1.4
NO-In	35.3±0.8	75.3±5.1	274.8± 11.0	649.9±11.1	2.7±0.2	9.7±0.3	22.9±0.8
NL-Out	23.6±2.6	92.8±6.5	233.2± 10.0	674.0±10.0	2.2±0.3	5.5±0.6	15.9±1.8
NL-In	25.9±4.0	57.6±8.0	253.8± 25.8	688.6±25.8	1.5±0.4	6.6±1.4	17.8±2.5
				q values			
NO-Out vs NO-In	**	***	*	ns	ns	***	**
NL-Out vs NL-In	ns	****	ns	ns	**	ns	ns

PL, phospholipids; GL, glycolipids; NO, *N. oceanica*; NL, *N. limnetica*; Out, grown outdoors; In, grown indoors; SD, standard deviation; ns; $q > 0.05$; *, $q < 0.05$; **, $q < 0.01$; ***, $q < 0.001$; ****, $q < 0.0001$.

The biomass content of PL, GL and neutral lipids plus pigments (expressed as mg/100 mg DW biomass) of the two *Nannochloropsis* species was also calculated and subjected to univariate analysis **(Table 5.1, Supplementary Table S5.3-Table S5.4**). These results showed that the biomass of *N. limnetica* grown indoors had the lowest PL content (1.5 ± 0.4 %), whereas the highest percentage was detected in *N. oceanica* grown outdoors and indoors (2.7 ± 0.2 %). *N. oceanica* grown indoors had the highest levels of GL (9.7 ± 0.3 %) and neutral lipids and pigments (22.9 ± 0.8 %). Interestingly, the amounts of PL, GL and neutral lipids plus pigments of the two species were significantly different only when grown in indoor photobioreactors (**Supplementary Table S5.3-Table S5.4**).

3.2. Fatty acid profile of *N. oceanica* and *N. limnetica* grown outdoors and indoors

The fatty acids (FAs) profile of lipid extracts of *N. oceanica* and *N. limnetica* grown outdoors and indoors was determined by GC-MS. Seventeen FAs were identified and quantified in absolute amounts (mg FA/100 mg lipid extract) (**Supplementary Table S5.5**) and in relative percentages of the total fatty acid pool (**Table 5.2**). The four most abundant FAs found in *N. oceanica* grown outdoors were C16:1 *n*-7, C20:5 *n*-3, C16:0, C20:4 *n*-6 (**Table 5.2**). The most abundant FAs found in *N. oceanica* grown indoors were C16:0, C16:1 *n*-7, C20:5 *n*-3, and C18:1 *n*-9. For this species, the growth indoors significantly increased C16:0 and C18:1 *n*-9 and decreased C16:1 *n*-7, C20:4 *n*-6, and C20:5 *n*-3. In *N. limnetica*, the FAs C20:5 *n*-3, C16:1 *n*-7, C16:0 and C20:4 *n*-6 were the most abundant outdoors, whereas indoors growth favoured the relative abundance of C16:0, C16:1 *n*-7, C20:5 *n*-3, and C18:1 *n*-9. Overall, indoor cultivation increased the relative abundance of C18:1 *n*-9 (3.2 ± 0.2 % versus 11.8 ± 0.8 % for NO and 4.3 ± 0.5 % versus 13.0 ± 1.6 % for NL) and decreased the relative abundance of C20:5 *n*-3 (28.9 ± 0.7 % versus 14.3 ± 2.2 % for NO and 33.1 ± 3.6 % versus 15.7 ± 1.4 % for NL).

To assess variations in the potential health benefits of lipid extracts, the thrombogenic (TI), atherogenic (AI) and hypocholesterolemic/hypercholesterolemic (h/H) indices were also determined (**Table 5.2**). TI and AI were lower in lipid extracts from the biomass grown outdoors, but statistically significant differences ($q < 0.01$) were observed only between lipid extracts from *N. oceanica*. TI values ranged from 0.2 ± 0.0 (NO Out) to 0.6 ± 0.1 (NO In) and AI values ranged from 0.5 ± 0.0 (NO Out) to 1.0 ± 0.2 (NO In). Lipid extracts of *N. oceanica* grown in outdoor photobioreactors had the highest h/H ratio (2.2).

Table 5.2. GC-MS analysis of the fatty acid composition of total lipid extracts of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In). Data are means ± SD, n=5.

	Mean ± SD				*q* values	
Fatty acid	NO-Out	NO-In	NL-Out	NL-In	NO-Out vs NO-In	NL-Out vs NL-In
C12:0	0.2±0.0	0.2±0.1	0.2±0.0	0.1±0.0	ns	*
C14:0	5.6±0.1	5.5±1.4	4.2±0.6	4.2±0.2	ns	ns
C15:0	0.2±0.0	0.6±0.1	0.3±0.0	0.2±0.0	**	ns
C16:0	**14.1±0.4**	**34.9±0.9**	**21.9±0.2**	**30.8±0.7**	**	ns
C16:1 Δ^{9} (*n*-7)	**33.4±0.5**	**25.0±1.0**	**26.8±3.1**	**24.6±0.1**	*	ns
C16:1 Δ^{7} (*n*-9)	0.3±0.0	0.1±0.0	0.3±0.1	0.1±0.0	ns	*
C16:2 $\Delta^{7,10}$ (*n*-6)	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	**	ns
C17:0	0.1±0.0	0.2±0.0	0.1±0.0	0.2±0.0	ns	**
C17:1 Δ^{8} (*n*-9)	0.1±0.0	0.3±0.0	0.1±0.0	0.2±0.0	*	*
C18:0	0.9±0.6	0.9±0.3	1.3±0.8	1.6±0.5	ns	ns
C18:1 Δ^{9} (*n*-9)	3.2±0.2	**11.8±0.8**	4.3±0.5	**13.0±1.6**	*	*
C18:1 Δ^{11} (*n*-7)	0.1±0.0	0.3±0.0	0.7±0.1	0.4±0.0	ns	ns
C18:2 $\Delta^{9,12}$ (*n*-6)	2.4±0.1	2.4±0.2	1.3±0.1	3.1±0.2	ns	**
C18:3 (*n*-6)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	*	ns
C20:3 (*n*-6)	0.6±0.1	0.1±0.0	0.2±0.1	0.2±0.1	**	ns
C20:4 (*n*-6)	**9.5±0.5**	3.4±0.5	**5.2±0.1**	5.3±0.5	**	ns
C20:5 (*n*-3)	**28.9±0.7**	**14.3±2.2**	**33.1±3.6**	**15.7±1.4**	*	*
∑ PUFA	**41.7±1.3**	20.3±2.8	**40.0±3.7**	24.6±0.8	**	ns
∑ (*n*-3)	**28.9±0.7**	14.3±2.2	**33.1±3.6**	15.7±1.4	*	*
∑ (*n*-6)	12.8±0.6	6.0±0.7	6.9±0.1	8.8±0.7	**	ns
∑ MUFA	37.2±0.5	37.5±0.4	32.2±3.4	38.2±1.6	ns	**
∑ SFA	21.1±0.9	**42.2±2.5**	27.8±0.7	37.2±0.9	**	ns
n-6/*n*-3 ratio	0.4±0.0	0.4±0.0	0.2±0.0	0.6±0.1	ns	**
h/H ratio	**2.2±0.1**	0.8±0.1	**1.7±0.2**	1.1±0.0	**	ns
AI	**0.5±0.0**	1.0±0.2	**0.5±0.0**	0.8±0.0	**	ns

TI	**0.2±0.0**	0.6±0.1	**0.2±0.0**	0.5±0.0	**	ns

The most abundant FA are highlighted in bold font. NO, *N. oceanica*; NL, *N. limnetica*; Out, grown outdoors; In, grown indoors; ∑ PUFA, sum of polyunsaturated fatty acids; ∑ MUFA, sum of mono-unsaturated fatty acids; ∑ SFA, sum of saturated fatty acids; h/H ratio, hypocholesterolemic/hypercholesterolemic indices; AI, atherogenic index; TI, thrombogenic index; SD, standard deviation; ns, $q > 0.05$; *, $q < 0.05$; **, $q < 0.01$.

The FAs identified in lipid extracts of *Nannochloropsis* species were assessed using principal component analyses (PCA) (**Figure 5.1-A**). PCA analysis showed discrimination among the four groups, but with some overlap between the two indoor samples (NO-In and NL-In). NO-Out versus NO-In and NL-Out versus NL-In were split along dim1 (53 % of variance), while NO-Out versus NL-Out and NO-In versus NL-In were split along dim2 (22.2 % of variance). These results showed a closer distance between different species grown under the same conditions than the same species grown under different conditions.

The two-dimensional hierarchical clustering heat map revealed the association of samples based on growing conditions (**Figure 5.1-B**). The FA data sets were grouped into two main clusters: the first group includes FAs that were more abundant in samples grown outdoors. This group was divided into two groups, one containing C16:1 *n*-9, and C20:5 *n*-3, which were most abundant outdoors, and the other containing, among others, C20:3 *n*-6, C20:4 *n*-6 and C16:2 *n*-6 higher in *N. oceanica* grown outdoors. The second group, which contained the most abundant FAs in indoor samples, had, among others, C16:0, and C18:1 *n*-9.

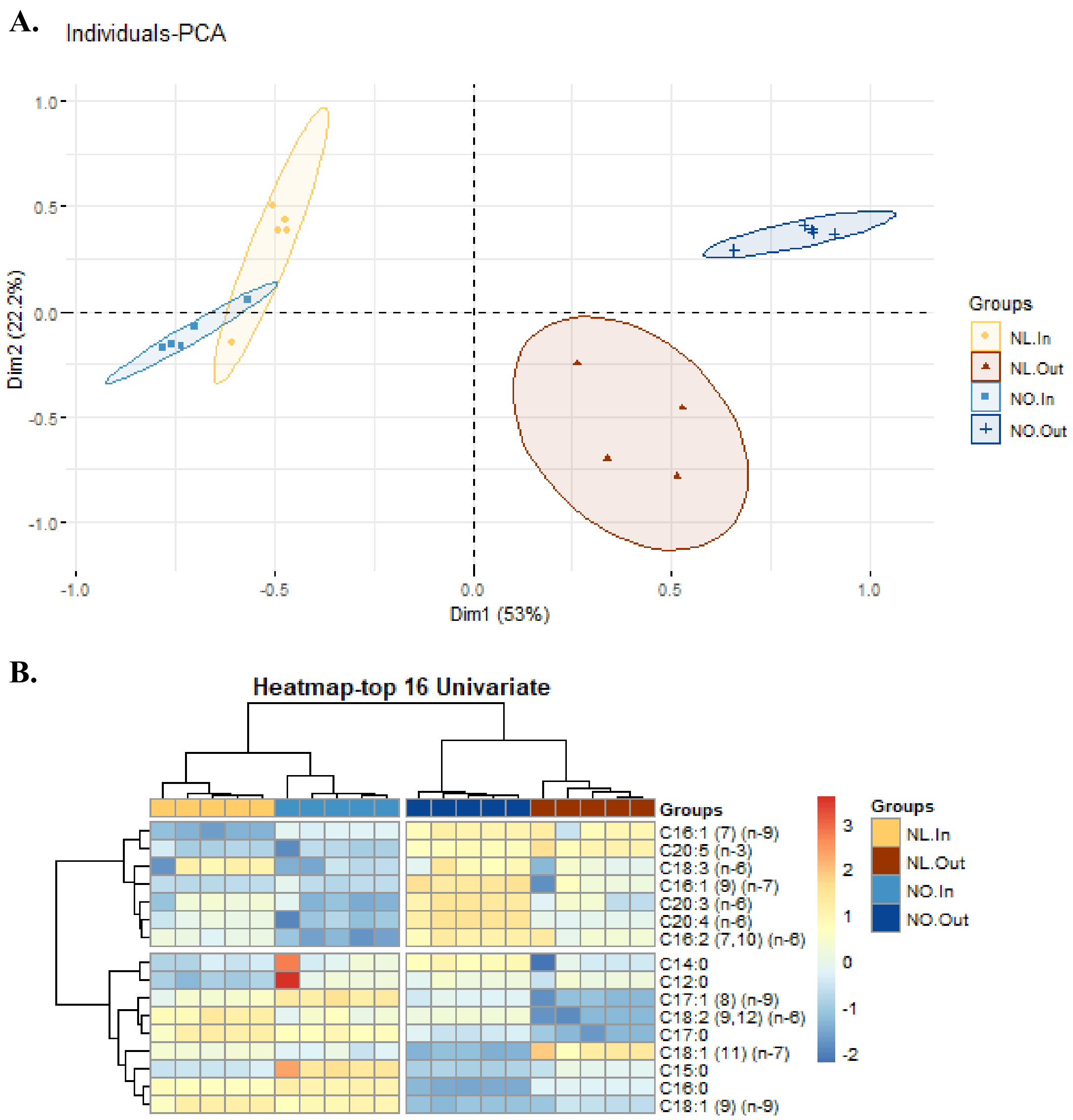

Figure 5.1. (**A**) Principal component analysis (PCA) score plot of GC-MS fatty acid data from *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In). (**B**) Two-dimensional hierarchical clustering heat map of statistically different fatty acids. Relative abundance levels are shown on the colour scale and numbers indicate the fold difference from the mean. The dendrogram at the top represents the clustering of the sample groups, showing four main clusters, one for each sample group. The dendrogram on the left represents the clustering of individual FAs given their similarity in relative abundance.

3.3. The polar lipid profile of *N. oceanica* and *N. limnetica* grown outdoors and indoors

The polar lipid profile of the total lipid extracts of the microalgal biomasses was analyzed and semi-quantified by HILIC-ESI-MS and MS/MS. We identified 232 (NO-Out), 231 (NO-In), 234 (NL-Out) and 233 (NL-In) lipid species in these lipid extracts. These species belong to four main classes of polar lipids, namely GL, PL, betaine lipids (BL) and sphingolipids (SL) (**Table 5.3**). The same classes of polar lipids were identified in all four groups and included phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI) and their lyso derivatives (LPC, LPE and LPG), diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine (DGTS) and their lyso form monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine (MGTS), sulfoquinovosyldiacylglycerol (SQDG), digalactosyldiacylglycerol (DGDG), digalactosylmonoacylglycerol (DGMG), monogalactosyldiacylglycerol (MGDG), monogalactosylmonoacylglycerol (MGMG), ceramide (Cer) and inositolphosphoceramide (PI-Cer). A complete list of lipid species and their accurate mass measurement is provided in **Supplementary Table S5.6.** The lipid molecular species are described in **Supplementary Table S5.7**. A list of the *m/z* values of the main fragment ions used to identify each lipid species and lipid molecular species is provided in **Supplementary Table S5.8-Table S5.11**. A number of 220 lipid species out of a total of 239 species identified were common to all groups (**Supplementary Figure S5.2**). A few specific lipids were highlighted, particularly two GLs that were specific to indoor cultivation (MGDG(34:2) and MGDG(34:1)), one PL to outdoor cultivation (PG(36:6)) and two lipid species were characteristic of *N. oceanica* grown outdoors (PI(28:0) and DGTS(36:3)).

Table 5.3. Classes of polar lipids identified in total lipid extracts of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In), and the number of lipid species identified in each class for each group.

	Number of lipid species			
Lipid classes	**NO-Out**	**NO-In**	**NL-Out**	**NL-In**
Glycolipids	40	45	43	45
DGDG	17	17	17	17
DGMG	4	4	4	4
MGDG	8	13	11	13
MGMG	1	1	1	1
SQDG	10	10	10	10
Phospholipids	138	132	138	134

LPC	20	20	20	20
LPE	13	13	13	13
PC	51	50	51	51
PE	28	28	29	29
LPG	1	1	1	1
PG	14	10	14	13
PI	11	10	10	7
Betaine lipids	50	50	50	50
DGTS	33	33	33	33
MGTS	17	17	17	17
Sphingolipids	4	4	3	4
PI_Cer	2	2	1	2
Cer	2	2	2	2
Total	232	231	234	233

Abbreviations: NO, *N. oceanica*; NL, *N. limnetica*; Out, grown outdoors; In, grown indoors; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

All identified lipid species were semi-quantified and subjected to statistical analysis (**Supplementary Figure S5.3–Figure S5.10 and Supplementary Table S5.12-Table S5.13**). The PCA analysis (**Figure 5.2-A**) showed a total variance of 84.1 % (dim1 49.2 %; dim 2 34.9 %). Separation of groups by growth conditions (In, Out) occurred along dim1, while species (NO, NL) were split mainly along dim2. These results also showed a smaller distance between different species grown under the same conditions than those grown under different conditions.

A two-dimensional hierarchical clustering heat map, shown in **Figure 5.2-B**, was constructed using the 50 lipid species with the lowest *q*-values (differences between the groups NL Out/NO Out and NL In/NO In were not considered). The top dendrogram clustered in the first leaf the samples into two main groups according to the growth conditions (NL-In and NO-In) and (NL-Out and NO-Out). The clustering of lipid species given their similarity in changes in relative lipid abundance represented by the dendrogram on the left of **Figure 5.2-B** showed two main clusters. The first cluster consisted of lipid species that were upregulated indoors (NO-In and NL-In) and downregulated outdoors (NO-

Out and NL-Out). This first group contained 23 lipid species [14 GL (10 MGDG, 3 DGDG, 1 MGMG), 6 PL (4 PC, 2 PE) and 3 BL (2 DGTS, and 1 MGTS)]. Of these, 9 species were esterified with EPA [MGDG(40:9); MGDG(32:5); MGDG(36:6); MGDG(36:5); MGDG(34:5); MGDG(38:7); PC(40:5); PE(38:6); DGDG(38:7)] and 7 with arachidonic acid (ARA) [MGDG(40:9); MGDG(36:5); MGDG(40:8); PC(40:5); PE(38:5); PE(38:6); DGTS(36:4)]. The second group contains lipid species upregulated outdoors (NO-Out and NL-Out) and consists of 27 lipid species [2 GL (1 SQDG, 1 DGDG), 16 PL (7 PC, 5 LPC, 3 PI, 1 PE) 9 BL (6 DGTS, 3 MGTS)]. Of these, 5 species were esterified with EPA [PI(36:6); PC(34:5); PC(38:9); DGTS(38:9); DGDG(40:10)]. Overall, GLs were upregulated under indoor conditions, while PLs and BLs were upregulated under outdoor conditions.

The heatmaps in **Supplementary Figure S5.11A and Figure S5.11B** show the 50 polar lipid species that contributed most to the separation of *Nannochloropsis* species grown outdoors (NL Out versus NO Out) and indoors (NL In versus NO In), respectively. When both species were grown outdoors (**Supplementary Figure S5.11A**), the polar lipidome of *N. oceanica* had 18 upregulated lipid species (11 BLs, 5 PLs, 1 GL, and 1 SL). Of these, the increase in DGTS and a bioactive sulphoglycolipid [SQDG(34:2)] is noteworthy. On the other hand, *N. limnetica* had 32 upregulated lipid species 20 PL, 6 GL and 6 BL. Of these, it is also noteworthy the higher amounts of lysolipids (LPC, LPE, DGMG, and MGTS) and bioactive glycolipids (MGDG(40:9), MGDG(40:10) and DGDG(36:5)). Interestingly, when both species were grown indoors (**Supplementary Figure S5.11B**), a similar trend was observed, with the polar lipidome of *N. oceanica* distinguished by enrichment in DGTS and SQDG, whereas *N. limnetica* had higher amounts of lysolipids (LPC and LPE) and bioactive GLs (MGDG(40:9) and MGDG(40:10)).

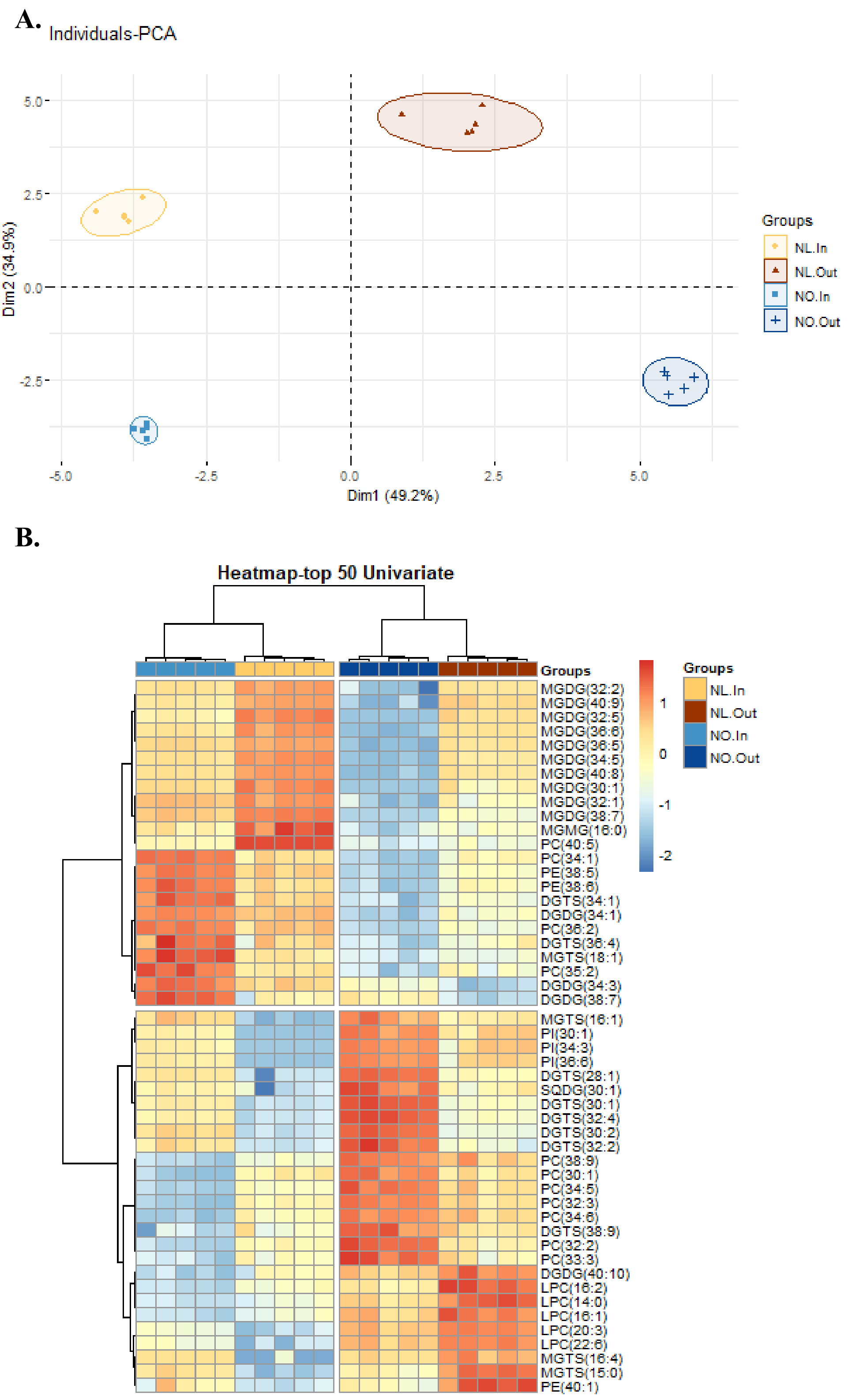

Figure 5.2. (**A**) Principal component analyses (PCA) scores plot of HILIC-ESI-MS polar lipid data of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In). (**B**) Two-dimensional hierarchical clustering heat map of the 50 most significant polar lipids (lower *q* values). Relative abundance levels are shown on the colour scale and numbers indicate the fold difference

from the mean. The dendrogram at the top represents the clustering of sample groups, showing four main clusters, one for each group. The dendrogram on the left represents the clustering of individual lipid species given their similarity in relative abundance. Abbreviations: MGDG, Monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine.

A pairwise comparison between outdoor and indoor cultivation for each *Nannochloropsis* species was performed to detect significant changes in polar lipid profiles. The comparison revealed significantly upregulated and downregulated lipids of *N. oceanica* grown outdoors and indoors (**Figure 5.3-A, Supplementary Table S5.14)**. This analysis showed 151 differentially regulated lipids out of a total of 239, of which 67 were upregulated under indoor conditions, and 84 were downregulated. Upregulated lipids of *N. oceanica* grown indoors were richer in longer FAs with higher degrees of unsaturation than those that were down-regulated. The volcano plot in **Figure 5.3-B** shows the significant differentially regulated polar lipids in *N. limnetica*. Of a total of 237 polar lipids, 119 were differentially regulated lipids, of which 38 were upregulated in the NL-In group, and 81 were downregulated (**Figure 5.3-B, Supplementary Table S5.15**).

Comparison of 25 major upregulated lipid species in *N. limnetica* and *N.oceanica* grown indoors (>2- fold) revealed that 13 were common to both species: 11 GLs [MGDG(40:8), MGDG(38:7), MGDG(36:5), MGDG(34:5), MGDG(34:2), MGDG(34:1), MGDG(32:2), MGDG(32:1), MGDG(30:1), DGDG(34:2), DGDG(34:1)] and 2 PLs [PC(38:1), PC(36:2)]. On the other hand, a comparison of 25 major upregulated lipid species of *N. limnetica* and *N.oceanica* grown outdoors (>2- fold) revealed that 8 PLs were common to both species: [PC(34:7), PC(36:8), PE(36:2), PG(36:6), LPC(17:0), LPE(16:3), PI(36:6), PI(34:3)]. Of these, PG(36:6) represented one of the five most abundant species in the two microalgae grown outdoors and was absent from the lipidome of the two microalgae grown indoors.

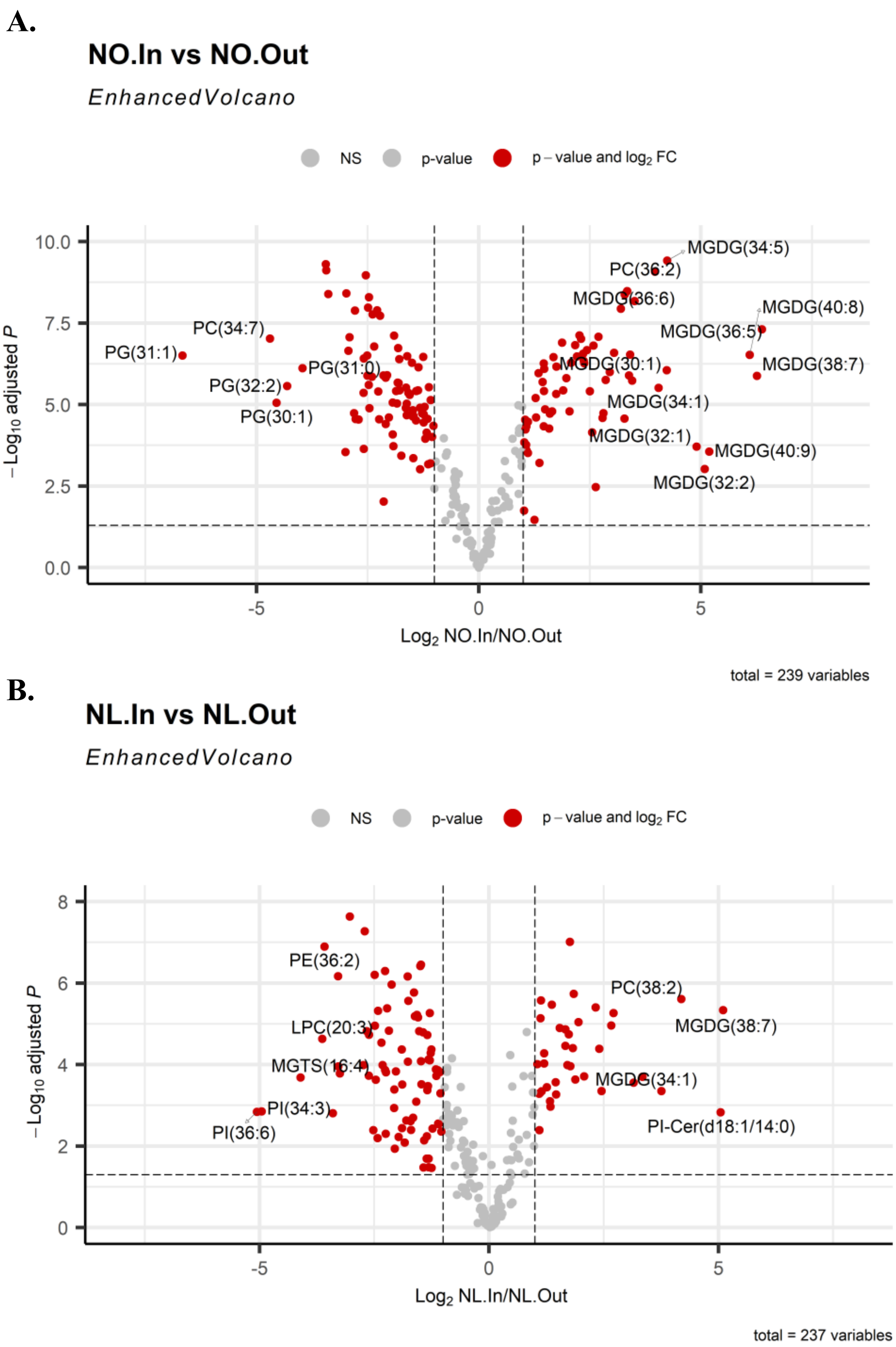

Figure 5.3. Volcano plots of pairwise comparisons of polar lipids of (**A**) *N. oceanica* (NO) grown outdoors (Out) versus indoors (In) and (**B**) *N. limnetica* (NL) Outdoors versus Indoors. Red dots show polar lipids with a significant fold change (>2- or <-2-fold) and Welch's t-test *p*-value (<0.05). Labelled lipids represent the most significant fold change (>11 or <-11). Abbreviations: MGDG, Monogalactosyldiacylglycerol; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; LPC, lysophosphatidylcholine; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; PG, phosphatidylglycerol and PI-Cer, inositolphosphoceramide.

Differences in the content of lipid classes (sum of normalized areas per lipid class) between groups were also assessed using univariate analysis (**Figure 5.4, Supplementary Table S5.16-Table S5.17**). There were significant differences in the amount of LPG ($q < 0.05$), PI ($q < 0.05$), PI_Cer ($q < 0.05$), and SQDG ($q < 0.01$) between species grown indoors (NL-In versus NO-In), all of which were more abundant in the lipid extracts of *N. oceanica*. On the other hand, the content of DGMG, LPC, and LPE varied significantly between species grown outdoors (NL-Out versus NO-Out), which were more abundant in the lipid extracts of *N. limnetica*.

There were significant differences in the amount of MGMG, MGTS, PC, PI, and DGDG between growth conditions (Out versus In). Samples of *N. limnetica* grown indoors were significantly more abundant in MGMG species ($q < 0.05$) and PC species ($q < 0.05$), and less abundant in MGTS species ($q < 0.01$) and PI species ($q < 0.05$) than in samples grown outdoors. Indoor-cultivated samples of *N. oceanica* were significantly more abundant in DGDG ($q < 0.05$) and MGMG ($q < 0.05$) than those grown outdoors.

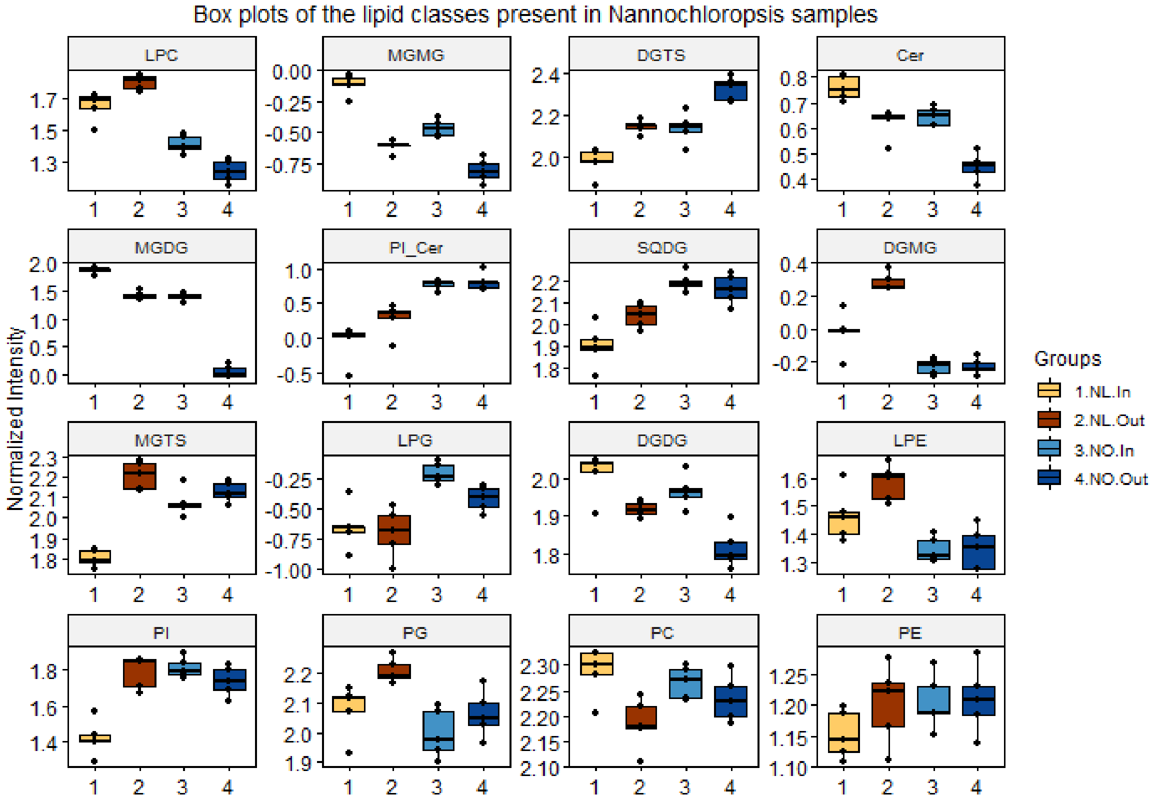

Figure 5.4. Box plots of lipid classes present in total lipid extracts of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC,

lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide, PI-Cer, inositolphosphoceramide.

3.4. Antioxidant activity of lipid extracts of *N. oceanica* and *N. limnetica*.

The *in vitro* antioxidant activity of lipid extracts of *N. oceanica* and *N. limnetica* grown outdoors and indoors was determined by the DPPH assay. The results showed that the lipid extracts of *N. oceanica* grown indoors had a lower inhibitory concentration (IC) than the same species grown outdoors (**Table 5.4**). Interestingly, extracts of *N. limnetica* showed the opposite pattern, with extracts from samples grown outdoors showing the highest activity (**Table 5.4**).

Table 5.4. Lipid extracts of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors providing 15 % inhibitory concentration (IC15) of DPPH radical scavenging activity and the corresponding Trolox Equivalent (TE). Data are means ± SD, n=3. Differences with a *q* value < 0.05 were considered statistically significant.

		Mean ± SD				*q*-value	
		NO Out	NO In	NL Out	NL In	NO Out vs NO In	NL Out vs NL In
DPPH •	IC15 (μg.mL^{-1})	229.0±14.3	151.9±5.3	175.5±1.8	225.9±9.1	****	**
	TE (μmol.g^{-1})	30.4±1.8	45.7±1.6	39.5±0.4	33.7±1.3	****	*

*q < 0.05; **q < 0.01; ****q < 0.0001

4. Discussion

The genus *Nannochloropsis* is a valuable source of polar lipids esterified with *n*-3 PUFAs, primarily EPA [13]. The polar lipidome of microalgae species is sensitive to fluctuations in environmental and growth conditions, which can be optimized to increase the production efficiency of healthy polar lipids [56]. In this work, we investigated the plasticity of the lipid composition of marine *N. oceanica* (NO) and freshwater *N. limnetica* (NL) grown under two cultivation conditions.

The results revealed a variation in lipid content depending on the species and growing conditions. *N. oceanica* grown indoors had the highest lipid content: 35 %, compared to 26 % obtained outdoors, while *N. limnetica* had 26 % and 24 %, respectively, although the latter values did not reach statistical significance. The range of lipid content observed in this study is consistent with the literature, reported between 24-53 % DW for *N. oceanica* and 24-41 % DW for *N. limnetica* [12,57]. These ranges are the result of the ability of microalgae to adapt to broad environmental conditions. Indeed, reports have shown that the lipid content of *Nannochloropsis* sp. is affected by various abiotic factors, including nitrogen concentration [58,59], pH [60], salinity [60], temperature [58,59,61,62] and light intensity [61,63].

One of the objectives of this work is related to establishing the growth conditions used in this study in an industrial setting. It is crucial to understand if the cultures obtained in the laboratory are comparable to those obtained under the conditions used for the production of industrial biomass, which has not yet been verified for the species studied in this work. During our study, the nitrogen concentration, pH and salinity were kept constant. However, while the indoor cultures were continuously exposed to 100 μmol photons.m^{-2} s^{-1} and 24 °C, these conditions were not controlled in the outdoor experiments. During the period of the experiment (January 7 - 27, 2020, Pataias, Portugal), the solar radiation varied between 0 and 500 w m^{-2}, and the local temperature ranged from 0 to 18 °C (**Supplementary Figure S5.1**). A combination of light and temperature could be responsible for the different lipid accumulation observed in our experiment.

It is common to find studies in the literature, corroborating these observations and reporting an increase in lipid content due to increased light intensity. An increase in illuminance from 1,000 to 2,000 lux in *N. gaditana* resulted in a 3.5 fold increase in lipid production [64]. It has also been reported that *N. oceanica* increases its lipid productivity with an increase in radiation from 100 to 800 μmol photons m^{-2} s^{-1} [65]. Other studies have reported an accumulation of lipids with an increase in temperature to the optimum value, for several microalgae including those of the genus *Nannochloropsis* (*Nannochloropsis* sp. [61] *N. oceanica* [62], *N. oculata* [59], *N. salina* [58]).

The amount of PLs and GLs was determined in the total lipid extracts, and their amount in the whole biomass was extrapolated. The biomass of *N. oceanica* grown indoors had the highest lipid content (**Table 5.1**, **Supplementary Table S5.2)**, the highest GL content, and a similar amount of PL as that detected grown outdoors (**Table 5.1**, **Supplementary Table S5.4**). However, different trends were observed in the lipid extract. For example, the GLs content in the lipid extract was increased by indoor cultivation, while outdoor growth increased the relative abundance of the PLs fraction. Fluctuations in PL and GL content have been previously reported in *Nannochloropsis* sp. [66]. The increase in GL content could result from higher photosynthetic activity due to constant exposure to light than in outdoor cultivation since GLs are the main components of the chloroplast [33].

The fatty acid (FA) profiles of *N. oceanica* and *N. limnetica* were recorded, and the same FAs were identified but with significant variations depending on the growth conditions. The main FAs for both species were C16:0, C16:1 *n*-7, and C20:5, which is consistent with the literature [4,67]. Outdoor growth showed increased levels of PUFAs. The concentration of EPA, reported in mg FA/100 mg lipid extract, ranged from 10.5 and 9.4 indoors to 14.3 and 13.3 outdoors in *N. oceanica* and *N. limnetica*, respectively. Another interesting observation was the increase in the amount of C18:1 FA in indoor lipid extracts, from 3.6 to 11.6 in *N. oceanica* and from 3.7 to 10.6 in *N. limnetica*, which may suggest an increase in *de novo* FAs synthesis [68]. Higher light intensities are associated with a reduction in the relative abundance of PUFAs [18,61,69,70]. Membrane fluidity is known to be regulated by this process, and similar trends have also been observed at higher temperatures [70,71]. The higher abundance of PUFAs and EPA in the biomass of N*annochloropsis* sp. grown outdoors could be due to a lower temperature and dark/light cycles in opposition to constant temperature and continuous light exposure indoors. *Nannochloropsis* species are considered sustainable producers of EPA, and variation in FA content modulates the nutritional value of the biomass. *N. oceanica* grown outdoors had the best nutritional quality indices (AI

(0.5±0.0), TI (0.2±0.0) and h/H ratio (2.2±0.1)). These values are similar to those reported in the literature, reinforcing the potential health benefits of this microalgae [72].

PUFAs are mainly found esterified in complex polar lipids of *Nannochloropsis* sp. PLs and GLs containing *n*-3 PUFAs are attracting interest for their bioactive properties, health benefits, and improved bioaccessibility of PUFAs, as reviewed by Haq and colleagues [73]. This work reports for the first time the polar lipidome of *N. limnetica*. It shares the same lipid classes and most polar lipid species as *N. oceanica*, but with differences in their relative abundances. The lipidomic characterization of the two microalgae showed a total of 239 lipid species from 16 classes, with three new classes reported here for the first time in *N. oceanica* (LPE, LPG, Cer) [29,30,74]. Of the 239 lipid species identified, 220 were shared between all groups. All lipid extracts were characterized with a higher number of PL species, followed by BL, GL and sphingolipids. Other works have also reported a higher number of PL than other lipid groups in *N. oceanica* [29,30], *Nannochloropsis* sp. [75,76] and *N. oculata* [26]. Three lipid species were specific to culture conditions: the GLs MGDG(34:1) and MGDG(34:2), which were found in the biomass grown indoors, and PG(36:6), which was specific to microalgae grown outdoors. This PG has been assigned as PG(20:5_16:1) and was previously associated with anti-inflammatory activity [77].

Additionally, the greatest difference between the groups was found in the amount of lipid species. Similar results have been reported for other species of algae (e.g. *Fucus vesiculosus* collected in different seasons [78]) and microalgae (e.g. *Chlorella vulgaris* grown in auto- and heterotrophy [33]). The content of almost all lipid species changes with growing conditions and between species of *Nannochloropsis*. For example, in *N. oceanica* and *N. limnetica* grown outdoors, the most abundant PC was 32:2 and 36:5 respectively, and when grown indoors it was 34:2. Although, the most abundant PC was the same for both species of *Nannochloropsis* grown indoors, they were in different amounts. Interestingly, it was possible to detect a clear distinction between the polar lipid profile of *Nannochloropsis* species, with a higher content of DGTS in *N. oceanica* and lysolipids (LPC and LPE) in *N. Limnetica*.

All groups showed significant fluctuations in the abundance of FAs and polar lipid species. PCA and heatmap analysis showed a closer relationship between samples from the same growth conditions. For example, using polar lipid profiles, growth conditions (In, Out) were separated along PC1 (49.2 %) while *Nannochloropsis* species (NO, NL) were separated along PC2 (34.9 %). The polar lipidome is highly sensitive and plastic and can be modulated according to growth conditions [33]. Lipidomics is also considered a chemotaxonomic tool capable of distinguishing morphologically similar microalgae [79,80], as shown in the current work with *N. oceanica* and *N. limnetica*.

The 50 most discriminating species of polar lipids, determined after univariate analysis, included 10 MGDG, 1 MGMG, 3 DGDG, 4 PC, 2 PE, 2 DGTS and 1 MGTS, upregulated in species grown indoors, and 6 DGTS, 3 MGTS, 5 LPC, 3 PI, 7 PC, 1 PE, 1 SQDG, 1 DGDG, upregulated outdoors. Interestingly, MGDG glycolipids, which are important constituents of chloroplast membranes, accounted for approximately 43 % of the upregulated lipid species in indoor conditions. However, a previous study reported a decrease in MGDG species in *N. oceanica* when exposed to high light intensity conditions [30]. However, the observed increase in MGDG could be a result of the higher indoor temperature, as lower temperatures have been described to decrease MGDG content [56]. On the other hand, DGTS accounted for approximately 22 % of upregulated species grown outdoors. Studies have reported an increase in DGTS in *N. oceanica* when deprived of inorganic phosphate (Pi) [81] and when subjected to low temperatures [56], which could explain the overall increase in DGTS when grown outdoors. Murakami et al. [56], found that in *Nannochloropsis* sp. at low temperature, an increase in DGTS species enriched in EPA is observed [56], which is in agreement with the results of this work. For example, the relative abundance of DGTS(40:10) esterified with EPA increased during outdoor growth. Interestingly, the same authors proposed a relationship between the abundances of DGTS and MGDG, which is consistent with this report: as the abundance of DGTS species increases, the abundance of MGDG species decreases.

Outdoor cultivation appeared to increase lyso lipids, such as MGTS and LPC, and PI species. An increase in these signalling molecules can be found in heterotrophic *C. vulgaris* compared to autotrophic conditions [33] and in plants under stress conditions [82,83]. This suggests that these algae grown outdoors are more susceptible to stress than those grown indoors.

The laboratory culture of the tested *Nannochloropsis* species provides more stable culture conditions, while the outdoor Flat Panels are exposed to the available environmental conditions, namely temperature and light irradiation [84]. It is known that growth conditions strongly modulate the polar lipidome of microalgae [33,56], promoting the variation in the relative abundance of bioactive polar lipids. Unravelling the regulation of lipid species is crucial for the added value of extracts and biomass. Indoor cultivation is usually the first step in the industrial cultivation of microalgae. Although some manufacturers produce their biomass indoors, the production costs are significantly higher than for outdoor cultivation. However, in this work, we found that lipid extracts of *N. oceanica* grown indoors had the best antioxidant potential, with similar antioxidant activity to that previously reported [72].

5. Conclusions

This study provides the first characterization of the polar lipidome of *N. limnetica*, contributing to the knowledge of its nutritional value and biotechnological potential. Additionally, we characterized the lipid metabolic adaptation of *Nannochloropsis* species grown under indoor controlled conditions and in outdoor photobioreactors. While indoor cultivation led to higher lipid productivity, particularly of glycolipids, outdoor growth promoted an increase in the proportion of phospholipids and betaine lipids esterified with EPA. Comparison of the polar lipid profile of the two *Nannochloropsis* species revealed that *N. oceanica* had higher amounts of some betaine lipids, while *N. limnetica* had higher amounts of lysolipids. Future studies should focus on seasonal variations in the polar lipidome of *Nannochloropsis* sp. grown outdoors. Overall, each microalga studied exhibited a specific polar lipid signature. It was shown that both species have the potential to be a source of healthy lipids of interest to the nutraceutical, food, pharmaceutical, and cosmeceutical industries. This work shows that indoor farming of *Nannochloropsis* species can be used to obtain lipids of interest with consistent and non-seasonal productivity, although at a higher production cost.

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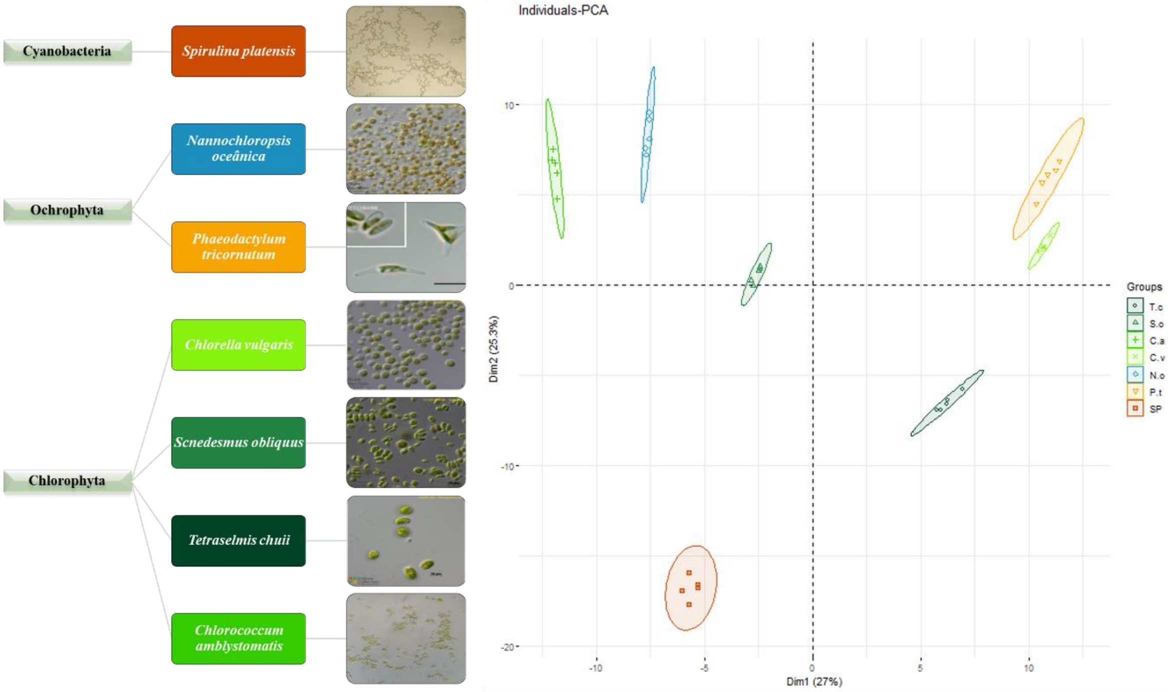
CHAPTER 6 - The chemodiversity of polar lipidomes of different taxa of commercial microalgae



Couto, D., Conde, T. A., Melo, T., Neves, B., Costa, M., Silva, J., Domingues, M. R. M., Domingues, P. The chemodiversity of polar lipidomes of different taxa of commercial microalgae. **Submitted in Algal Research in September 2022.**

The chemodiversity of polar lipidomes of different taxa of commercial microalgae

Abstract: Polar lipids from microalgae are attracting increasing interest due to their structural diversity, high value, and beneficial health effects. However, the relationship between lipid composition and biological diversity, as well as the potential applications of high-value microalgae lipids need to be further explored. In this study, we characterized and compared the polar lipidome of a taxonomically diverse set of 7 species of microalgae belonging to the phyla Chlorophyta (*Chlorella vulgaris*, *Scenedesmus obliquus*, *Tetraselmis chuii*, *Chlorococcum amblystomatis*), Ochrophyta (*Nannochloropsis oceanica*, *Phaeodactylum tricornutum*) and Cyanobacteria (*Spirulina* sp.), using high-resolution LC-MS analysis, to assess the chemotaxonomic specificity of polar lipids. 498 different species of polar lipids have been identified, including 109 common to all microalgae. Each microalga had a species-specific polar lipid signature but with a trend that reflected their phylogenetic relationship, allowing for possible distinction at lower taxonomic levels. Chloroplast-abundant lipids were the lipid species that contributed the most to discrimination between microalgae phyla, while betaine lipids were less reflective of the phylogenetic relationship. The specificity of the lipidome of microalgae favours the use of selected microalgae as bio-factories of specific lipids with high added value with a multitude of biological, sustainable, and ecological applications.

Keywords: Microalgae, Lipidomics, Polar Lipids, Phospholipids, Glycolipids, Chemotaxonomy

1. Introduction

Microalgae have a unique and valuable biochemical composition, rich in lipids, which favours their exploitation for different applications (*e.g.* food, feed, nutraceutical and pharmaceutical). They can be cultivated sustainably, without competing with other food crops for water or arable land. They may also be a sustainable alternative to fish as sources of essential and bioactive lipids with high content in ω-3 polyunsaturated fatty acids (PUFAs), with the potential to be used for nutraceuticals and supplements for a population with special needs (*e.g.,* the elderly and children), as well as for vegetarian and vegan diets. Indeed, there is growing interest in algae and algae-derived products for food target markets and novel ingredients for traditional products such as cookies and bread [1]. Microalgae have been considered the sustainable food of the future and some species are already approved by the European Food Safety Authority for human consumption such as *Chlorella* sp., *Spirulina* sp., *Euglena gracilis* and *Tetraselmis chuii* [2,3]. Other microalgae have been widely used as whole biomass in aquacultures such as *Tetradesmus* sp. (formerly *Scenedesmus* sp.) or *Tetraselmis* sp., but with a much lower market value [3,4]. Others have been explored to obtain dietary nutrients such as lipid extracts rich in ω-3 PUFAs for foods and feed supplements such as *Nannochloropsis* sp. and *Phaeodactylum tricornutum* [3].

Microalgae are an extremely diverse group, distributed in different ecosystems and with a wide taxonomic distribution. According to the latest classification model, microalgae include organisms from the superkingdoms Prokaryota (1 phylum Cyanobacteria), and Eukaryota (10 phyla) [5,6]. This diversity of microalgae encompasses a vast biochemical diversity and an opportunity for industrial exploitation. Although the molecular composition varies from species to species [7], it can be specific enough to distinguish between different strains [8,9]. While some compounds are species-exclusive (*e.g.,* proteins, polysaccharides) [10], others appear to be phylum-specific (*e.g.,* chlorophylls, phycobiliproteins, and carotenoids) [3]. Parameters such as the carbon to nitrogen (C:N) ratio can also predict phylogenetic classification at the microalgae phylum level. For example, Cyanobacteria have the lowest C:N ratio while Myzozoa has the highest [11,12]. Some studies have also reported that fatty acid (FA) profiles of microalgae [13–17] and macroalgae [18] reflect phylogenetic relationships at higher-level taxonomic ranks, such as phyla [13–16] and class [13,16,17], but not at lower taxa [13]. However, FAs in their free form are less abundant, and they are mainly esterified in complex lipids such as polar lipids (phospholipids [PLs], glycolipids [GLs] and betaine lipids [BLs]). Although polar lipids are one of the most abundant and diverse group of lipids in microalgae, their potential as a chemotaxonomic tool and as sources of valuable ingredients remains scarcely studied. Additionally, it is not known to what extent lipid profiles are specific to microalgae species or taxa. Only two studies involving algae demonstrated the relationship between algae polar lipids and taxonomic differentiation [19,20]. Cañavate *et al.* suggested that the combination of PLs and BLs was an optimal biomarker to discriminate microalgae at the class level [20]. However, these results were observed at the lipid class level without specific information at the molecular level [20].

The polar lipidome of most microalgae remains uncharacterized, which hinders the exploitation of these microalgae for the prospecting of bioactive lipids. Of the 200,000 to 800,000 known species [21], only about fifty species of microalgae have their polar lipid profile described [22]. The characterization of the polar lipidome of each microalga is challenging and the plasticity of polar lipids when microalgae are grown under different biotic and abiotic factors, such as temperature and salinity, may weaken the role of polar lipids in taxonomic classification [20]. Moreover, the data available in the literature were acquired in different laboratories using different extraction methodologies, instrumental conditions, and data acquisition parameters making it difficult to accurately compare the polar lipid profile of microalgae.

The present study tested the hypothesis that the polar lipid signature of microalgae is significant in a species-level phylogenetic context, and it may reflect their ecological niche. To test this hypothesis, the polar lipidome of a taxonomically diverse set of 7 of the most commercially available species of microalgae were characterized and compared using hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-MS). These species included the phyla Chlorophyta (*Chlorella vulgaris, Scenedesmus obliquus, Tetraselmis chuii*, *Chlorococcum amblystomatis*), Ochrophyta (*Nannochloropsis oceanica*, *Phaeodactylum tricornutum*) and Cyanobacteria (*Spirulina* sp.). This selection includes freshwater microalgae (*C. vulgaris*, *S. obliquus*, *C. amblystomatis,* and *Spirulina* sp.) and marine microalgae (*T. chuii*, *N. oceanica*, and *P. tricornutum*).

2. Materials and methods

2.1. Microalgae strain and culture media

Spray-dried biomass of all microalgae species (Chlorophyta (*Chlorella vulgaris, Scenedesmus obliquus*, *Tetraselmis chuii*, *Chlorococcum amblystomatis*), Ochrophyta (*Nannochloropsis oceanica*, *Phaeodactylum tricornutum*) and Cyanobacteria (*Spirulina* sp.) were provided by Allmicroalgae Natural Products S.A., located in Pataias, Portugal. All species were grown autotrophically using Guillard's F2 culture medium adapted to local water [14]. *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Tetraselmis chuii* were supplemented with magnesium mixture (Necton, Olhão, Portugal) and NaCl (Salexpor, Coimbra, Portugal) at 30 g/L salinity. Sodium bicarbonate (Quimitécnica, Barreiro, Portugal) at 16.8 g/L was added to the *Spirulina* sp. cultures. The microalgae cultures were cultivated in 5-L flask reactors under continuous 700 µmol photons.m^2/s light exposition from 7 to 15 days. Five 5-L flask reactors were used to inoculate one 0.1 m^3 L outdoor flat panel (FP) reactor, which was later scaled up to 1 m^3 FPs. Of the five FPs, four were used as inoculum of a 10 m^3 tubular photobioreactor (PBR), except for *Spirulina* sp., which was collected from the FPs. The reactor was exposed to ambient light and temperature conditions until the stationary phase was reached. A sprinkler-like irrigation system was used to keep the temperature of the PBR below the maximum limit. Pulse injections of CO_2 were used to keep the pH constant. The temperature limit and pH conditions in the 10 m^3 PBRs were operated as previously described [14]. The biomass was collected by centrifugation and dried by spray drying.

2.2. Reagents

HPLC grade dichloromethane (CH_2Cl_2), and methanol (CH_3OH) were purchased from Fisher Scientific Ltd. (Loughborough, UK). The water was of Milli-Q purity (Synergy1, Millipore Corporation, Billerica, MA, USA). All other reagents were purchased from major commercial sources. Lipid internal standards were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA): 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (dMPC), 1-

nonadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC), 1,2-dimyristoyl-*sn*-glycero-3-phospho-(10-rac-glycerol) (dMPG), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (dMPE), N-heptadecanoyl-D-erythro-sphingosine (Cer(17:0/d18:1)).

2.3. Extraction of total lipids

Extraction of lipids from *Scenedesmus obliquus*, *Chlorella vulgaris*, *Chlorococcum amblystomatis*, *Phaeodactylum tricornutum*, *Tetraselmis chuii*, *Nannochloropsis oceanica*, and *Spirulina* sp. was performed using Folch extraction method as detailed previously [14]. A solution of CH_2Cl_2:CH_3OH (2:1, v/v) was used to extract 25 mg of biomass. The suspension was vortexed for 2 min, centrifugated (2000 rpm for 10 min) and the organic phase was collected. This process was performed four times (4 × 2 mL). The organic phases were dried under a nitrogen stream and re-extracted with 2 mL of CH_2Cl_2, 1 mL of CH_3OH and 0.75 mL of Milli-Q water. Phase separation was obtained by centrifugation (2000 rpm for 10 min), and the organic phases were collected. The aqueous phase was re-extracted twice with 2 mL of CH_2Cl_2. The combined organic phases were dried and weighed. Five technical replicates were carried out for each microalga.

2.4. Hydrophilic interaction liquid chromatography-mass spectrometry (HILIC–ESI–MS)

The polar lipidome of the microalgae species was characterized using hydrophilic interaction liquid chromatography (HILIC) in a Dionex Ultimate 3000 (Thermo Fisher Scientific, Bremen, Germany) using an Ascentis® Express column (10 cm x 2.1 mm, 2.7 µm; Sigma-Aldrich) coupled to the Q-Exactive® hybrid quadrupole Orbitrap mass spectrometer (Thermo Fisher, Scientific, Bremen, Germany). The two mobile phases were H_2O/ CH_3CN/ CH_3OH (25:50:25, v/v/v) with 5 mM ammonium acetate (mobile phase A) and CH_3CN/CH_3OH (60:40, v/v) with 5 mM ammonium acetate (mobile phase B). The gradient started at 95 % B (0-2 min), followed by a linear decrease to 52 % B in 8 min, and a further linear decrease to 35 % B in 5 min, followed by a 2 min maintenance period and return to initial conditions in 3 min and re-equilibration of the column (10 min). Samples were prepared by mixing 20 µL of each sample (equivalent to 40 µg of lipid extract), 72 µL of eluent (95 % of B and 5 % of A), and 8 µL of lipid standards mixture (dMPC - 0.04 µg, LPC - 0.04 µg, dMPE - 0.04 µg, dMPG - 0.024 µg and Cer(17:0/d18:1) - 0.04 µg). 5 µL of this mixture was injected into the Ascentis® Express microbore column (10 cm x 2.1 mm, 2.7 µm; Sigma-Aldrich) at 35 °C and a flow-rate of 200 µL min^{-1}. The mass spectrometer operated simultaneously in positive (ESI 3.0 kV) and negative (ESI −2.7 kV) modes, with automatic gain control (AGC) target of 1×10^6 and high resolution (70,000). The sheath gas flow rate was 20 U, and the capillary temperature was 250 ºC. MS/MS determinations were performed as described in [23] with collision energy™ (CE) between 25, 30, and 35 eV and a resolution of 17500.

Data acquisition was carried out using the Xcalibur data system (V3.3, Thermo Fisher Scientific, Waltham, MA, USA). The identification of molecular species of polar lipids was based on LC retention time, mass accuracy (< 5 ppm), and detailed structural information inferred by MS/MS data, as reported previously [22,24–26]. Integration of the ion peak area was performed using the MZmine software package (version 2.53) and included baseline correction, peak deconvolution, deisotoping and alignment, and gap-filling [27]. Peaks with raw intensity lower than 10^4 were excluded. Normalization was performed by dividing the integrated peak areas of each molecular ion by the peak area of the selected internal standards. The amount of each group of lipids (PLs, GLs, BLs and SLs) was estimated by the sum of all lipid species belonging to the corresponding lipid group. The amount of each polar lipid class was estimated by the sum of all lipid species belonging to the corresponding lipid class. The relative abundance of lipid species, lipid groups, and lipid classes was used for statistical analysis.

2.5. Statistical Analysis

Multivariate and univariate analyses of data were performed using the R version 4.0.2 [28] in Rstudio version 1.3.1093 software [29]. All data sets (lipid species, lipid classes and lipid groups) were log-transformed. PCA and ellipses were created using R libraries FactoMineR [30] and factoextra [31]. Partial Least Squares-Discriminant Analysis (PLS-DA) was performed using the Metaboanalyst software [32]. A one-way ANOVA test followed by Tukey's multiple comparison test was performed using the R package rstatix [33]. Heatmaps and Hierarchical Cluster Analysis were created using the R package pheatmap using "Euclidean" as clustering distance and "ward.D" as the clustering method [34]. Heatmaps were constructed based on the 50 most significant polar lipids (lowest *p*-values in One-way ANOVA test), discriminating all microalgae, by marine and freshwater microalgae, and by phylum. The boxplots were created using the R package ggplot2 [35].

3. Results

3.1. Polar lipid composition of microalgae

The polar lipid signatures of *T. chuii*, *S. obliquus*, *P. tricornutum*, *C. vulgaris*, *C. amblystomatis*, *N. oceanica*, and *Spirulina* sp. were identified using HILIC-ESI-high resolution-MS and MS/MS. We have identified four main groups of polar lipids: glycolipids (GLs), phospholipids (PLs), betaine lipids (BLs) and sphingolipids (SLs), divided into 19 classes of polar lipids (**Table 6.1**). These included a total of 498 lipid species (**Table 6.1, Supplementary Table S6.1**): 407 in *P. tricornutum*, 341 in *C. amblystomatis*, 323 in *T. chuii*, 318 in *C. vulgaris*, 311 in *S. obliquus,* 301 in *N. oceanica*, and 205 in *Spirulina* sp. For all microalgae, PLs had the highest number of lipid species varying between 87 (*Spirulina* sp.) and 197 (*P. tricornutum*), followed by BLs, ranging between 57 (*C. vulgaris*) and 105 (*P. tricornutum*), and GLs, varying between 53 (*Spirulina* sp.) and 104 (*P. tricornutum*). Regarding lipid classes, some were absent in some species. For example, SQMGs were not found in *T. chuii*, *S. obliquus*, *C. vulgaris* and *N. oceanica.* Other classes were only found in some species, such as DGTA in *T. chuii*, *S. obliquus*, *N. oceanica* and *P. tricornutum,* and MGTA in *T. chuii* and *P. tricornutum*. LPE and LPG have been identified in all microalgae except *Spirulina* sp. and *T. chuii,* respectively. Regarding SL lipids, PI-Cer was not observed in *T. chuii* and *S. obliquus* and Cer was not detected in *T. chuii, P. tricornutum* and *Spirulina* sp.

Table 6.1 also shows, for the first time, the identification of lipids of different lipid classes in the microalgae studied: PC, LPC, PE, DGTS, MGTS and SQMG in *Spirulina* sp.; MGTS, MGTA, LPC, LPE and LPG in *P. tricornutum*; DGMG, MGMG, DGTS, MGTS, DGTA, MGTA, PC, LPC, PE, LPE, PI in *T. chuii* and DGDG, DGMG, MGMG, DGTS, MGTS, DGTA, LPE and Cer in *S. obliquus*. Also, we identified new lipid species and detected new lipid classes on the lipidome of the three species of microalgae previously reported by our laboratory [24,36,37], namely, DGTS, MGTS, and LPG in *C. vulgaris*, DGTA in *N. oceanica,* and LPG and Cer in *C. amblystomatis* (**Supplementary Table S6.1**).

Table 6.1. The number of lipid species per class of polar lipids identified in the total lipid extracts of *Tetraselmis chuii*, *Scenedesmus obliquus*, *Chlorococcum amblystomatis*, *Chlorella vulgaris*, *Nannochloropsis oceanica*, *Phaeodactylum tricornutum*, and *Spirulina* sp.

		Number of lipid species						
		Chlorophyta				**Ochrophyta**		**Cyanobacteria**
		T. chuii	*S. obliquus*	*C. amblystomatis*	*C. vulgaris*	*N. oceanica*	*P. tricornutum*	*Spirulina sp.*
Glycolipids (GL)	DGDG	29	25	27	24	21	25	14
	DGMG	5	5	11	4	5	8	5
	MGDG	25	20	27	19	23	34	12
	MGMG	7	5	12	4	7	9	6
	SQDG	18	18	22	18	13	27	14
	SQMG	0	0	1	0	0	1	2
	Total	**84**	**73**	**100**	**69**	**69**	**104**	**53**
Betaine lipids	DGTS	40	49	57	42	52	45	45
	MGTS	10	16	21	15	17	12	18
	DGTA	35	7	0	0	1	40	0
	MGTA	6	0	0	0	0	8	0
	Total	**91**	**72**	**78**	**57**	**70**	**105**	**63**
Phospholipids (PL)	PC	68	63	64	72	63	76	34
	LPC	16	15	20	22	19	27	7
	PE	35	33	25	42	27	52	20
	LPE	7	14	12	14	15	17	0
	PG	18	35	32	31	26	21	22
	LPG	0	1	1	2	1	1	2
	PI	4	4	5	6	7	3	2
	Total	**148**	**165**	**159**	**189**	**158**	**197**	**87**
Sphingolipids	PI-Cer	0	0	2	2	2	1	2
	Cer	0	1	2	1	2	0	0
	Total	**0**	**1**	**4**	**3**	**4**	**1**	**2**
Total lipid species		**323**	**311**	**341**	**318**	**301**	**407**	**205**

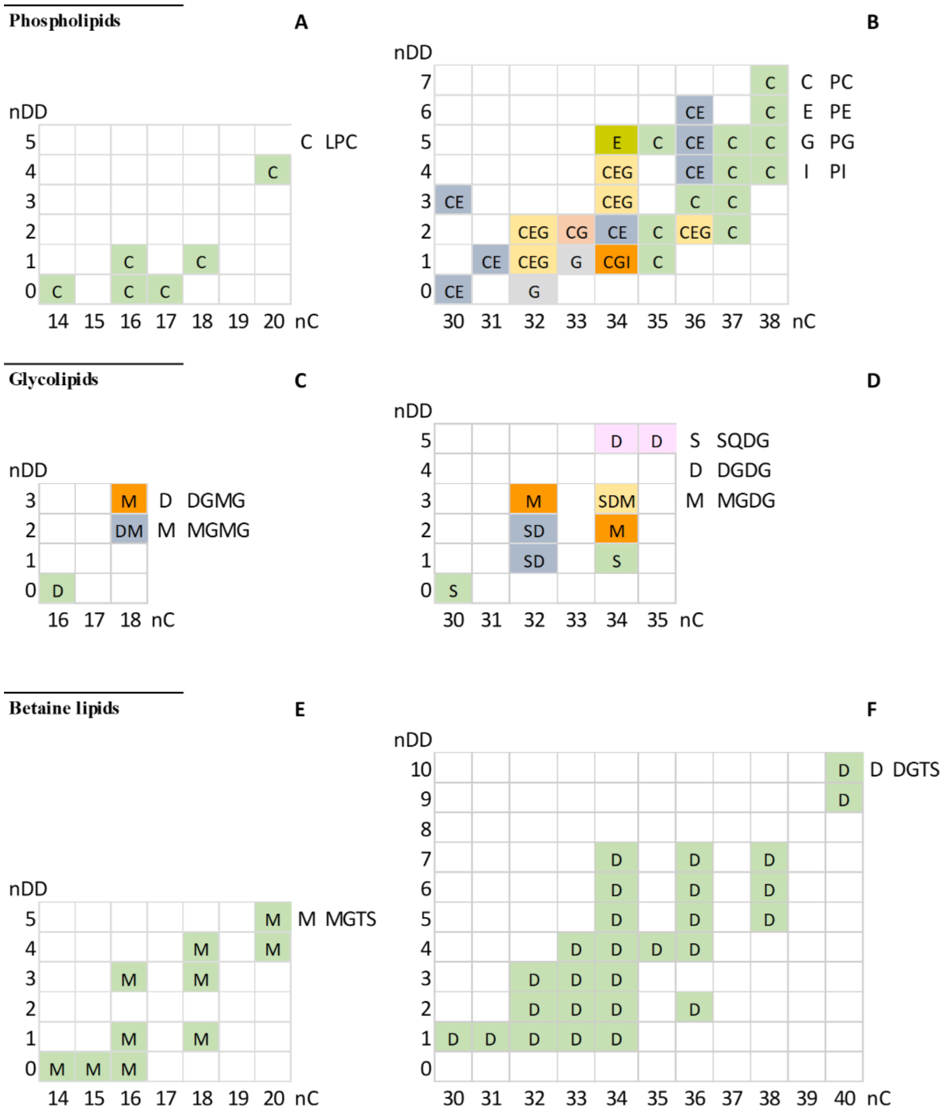
Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide

The comparison between the lipid profiles of all microalgae showed that 109 lipid species, out of 498 identified, were common to all microalgae, as illustrated in **Table 6.2**. These 109 lipid species correspond to 55 PLs (26 PC, 6 LPC, 13 PE, 9 PG, 1 PI), 37 BLs (27 DGTS, 10 MGTS), and 17 GLs (5 DGDG, 2 DGMG, 3 MGDG, 2 MGMG, 5 SQDG) lipid species (**Table 6.3**). The fatty acyl chain composition of these lipid species was identified for most species, and a similar composition in FA between microalgae was observed (**Supplementary Table S6.2**).

Table 6.2. The number of common polar lipid species identified in the total lipid extracts of *Tetraselmis chuii* (T.c), *Scenedesmus obliquus* (S.o), *Chlorococcum amblystomatis* (C.a), *Chlorella vulgaris* (C.v), *Nannochloropsis oceanica* (N.o), *Phaeodactylum tricornutum* (P.t) and *Spirulina* sp. (SP).

	Chlorophyta				Ochrophyta		Cyanob.
	C.v.	**S.o**	**T.c.**	**C.a.**	**N.o.**	**P.t.**	**SP**
C.v.	**318**	260	235	252	232	275	180
S.o		**311**	243	259	221	258	160
T.c.			**323**	242	211	293	154
C.a.				**341**	275	280	176
N.o.					**301**	264	161
P.t.						**407**	167
SP							**205**
Within Phylum	**193**				**264**		
Between Phylum	**160**						
Total	**109**						

Table 6.3. Common lipid species identified in all phyla. **A**, **B**) phospholipids; **C**, **D**) Glycolipids; **E**, **F**) Betaine lipids. For better visualization of the data, Excel colour scale conditional formatting was applied.



Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol. nDD, total number of double bonds; nC, total number of carbon atoms on the esterified fatty acids.

Chlorophyta microalgae shared 41% of lipid species, *i.e.*, 193 common lipid species (**Table 6.2**). Of 468 identified species, 55% were PLs, 25% were GLs and 20% were BLs (**Supplementary Table S6.3**). In Chlorophyta, certain lipid species were exclusive for each microalga: 45 lipids in *T. chuii* (35 BLs, 5 GLs and 5 PLs), 7 lipids in *S. obliquus* (4 GLs, 2 PLs and 1 BL), 40 lipids in *C. amblystomatis* (23 GLs, 7 BLs, 6 PLs and 4 SLs), and 24 lipids in *C. vulgaris* (16 PLs, 4 GLs, 2 BLs and 2 SLs). Species belonging to the phylum

Ochrophyta shared 264 lipids (59%) out of a total of 444 (**Table 6.2**), most of them being PLs (56%), followed by GLs (23%) and BLs (21%) (**Supplementary Table S6.4**). *N. oceanica* showed 37 unique species, which included 15 BLs, 11 PLs, 8 GLs and 3 SL lipids, while *P. tricornutum* had 143 unique species (50 PLs, 50 BLs and 43 GLs).

A total of 193 lipids were common to the phylum Chlorophyta and 264 lipids were common to the phylum Ochrophyta (**Table 6.2**). Chlorophyta and Ochrophyta microalgae shared a total of 160 (35 %) common lipids out of a total of 457 common lipids (**Table 6.2**). Freshwater microalgae (*Spirulina* sp., *S. obliquus*, *C. vulgaris* and *C. amblystomatis)* shared 143 (33%) lipids (45% PLs, 22% GLs, 33% BLs) out of a total of 429 (**Supplementary Table S6.5**), while saline microalgae (*T. chuii*, *N. oceanica* and *P. tricornutum)* shared 199 (43%) lipids (56% PLs, 22% GLs, 22% BLs) out of a total of 462 (**Supplementary Table S6.6**). Freshwater and saltwater microalgae shared 109 lipids, 34 were exclusive to freshwater microalgae, while 90 lipids were exclusive to saltwater microalgae.

3.2. Variations in the polar lipidome of microalgae

All lipid species identified in different microalgae were semi-quantified and compared. Discrimination according to the relative abundance of each lipid species was assessed using multivariate analysis. Principal component analysis (PCA) was performed using all 498 identified lipid species (**Figure 6.1**) and showed a clear separation between the polar lipid profile of each microalga.

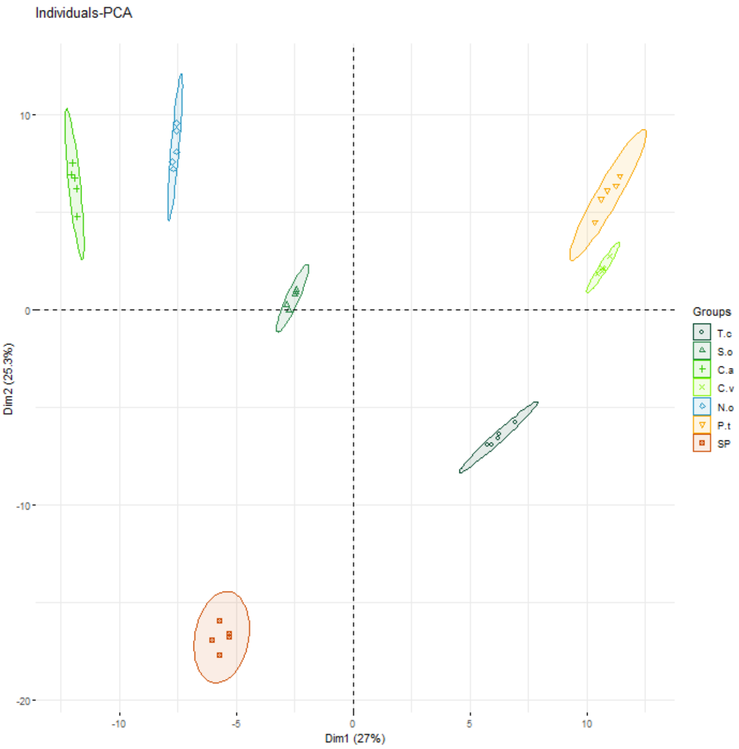


Figure 6.1. Principal component analysis (PCA) scores plot of HILIC-ESI-MS polar lipid data from *Tetraselmis chuii* (T.c), *Scenedesmus obliquus* (S.o), *Chlorococcum amblystomatis* (C.a), *Chlorella vulgaris* (C.v), *Nannochloropsis oceanica* (N.o), *Phaeodactylum tricornutum* (P.t) and *Spirulina* sp. (SP).

The heatmap plotted in **Figure 6.2** reveals the 50 most significantly different polar lipid species (lowest *p*-values) between different microalgae. The dendrogram at the top shows that the microalgae have been grouped by species but not by phylum or marine or freshwater origin. The dendrogram on the left consists of four main groups: the first group comprised of upregulated lipid species in *C. amblystomatis* and *S. obliquus*, and includes 15 BLs [14 DGTS and 1 MGTS]; the second group showed upregulated lipid species in *C. vulgaris* and consists of 8 lipids [6 PL (3 PE; 2 PG; 1 PC) and 2 GL (2 MGDG)]; the third group included upregulated lipid species in *T. chuii* and *P. tricornutum* and includes 14 lipid species [12 BL (12 DGTA) and 2 GL (1 MGDG and 1 SQDG)]; the fourth group showed significant lipids with high variability between microalgae.

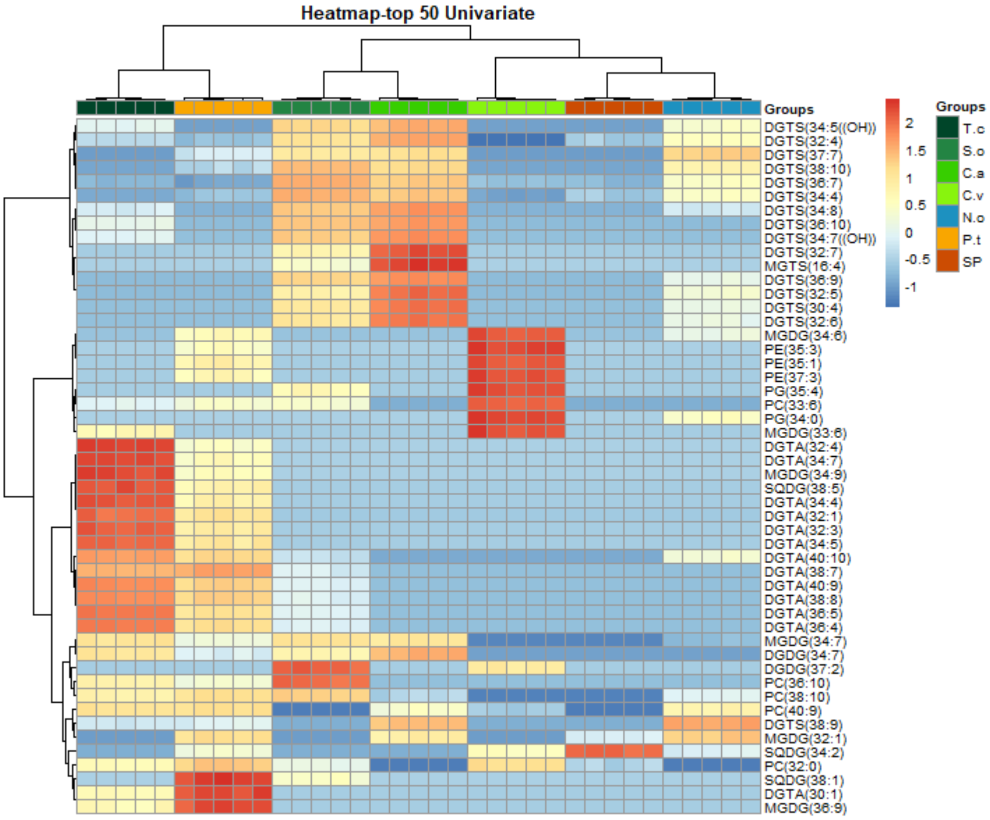


Figure 6.2. Two-dimensional hierarchical clustering heatmap of the 50 most significant polar lipids discriminating microalgae (*Chlorella vulgaris* (C.v), *Scenedesmus obliquus* (S.o), *Chlorococcum amblystomatis* (C.a), *Tetraselmis chuii* (T.c), *Nannochloropsis oceanica* (N.o), *Phaeodactylum tricornutum* (P.t), and *Spirulina* sp. (SP)). The relative abundance levels are indicated on the colour scale, and the numbers indicate the fold difference from the mean. The dendrogram at the top represents the clustering of sample groups, showing seven main clusters, one for each microalga. The dendrogram on the left represents the clustering of individual lipid species given their similarity in relative abundance.

A prediction model based on Partial Least Squares-Discriminant Analysis (PLS-DA) was then developed to classify freshwater and saltwater species from lipid data (**Figure 6.3**). Distinct clustering between classes was obtained from the first component with an accuracy=1, R^2=0.91, Q^2=0.97. The most important variables in the PLS-DA model (VIP score) included 6 betaine DGTA lipids, and 4 PC lipids, all more abundant in saltwater species.

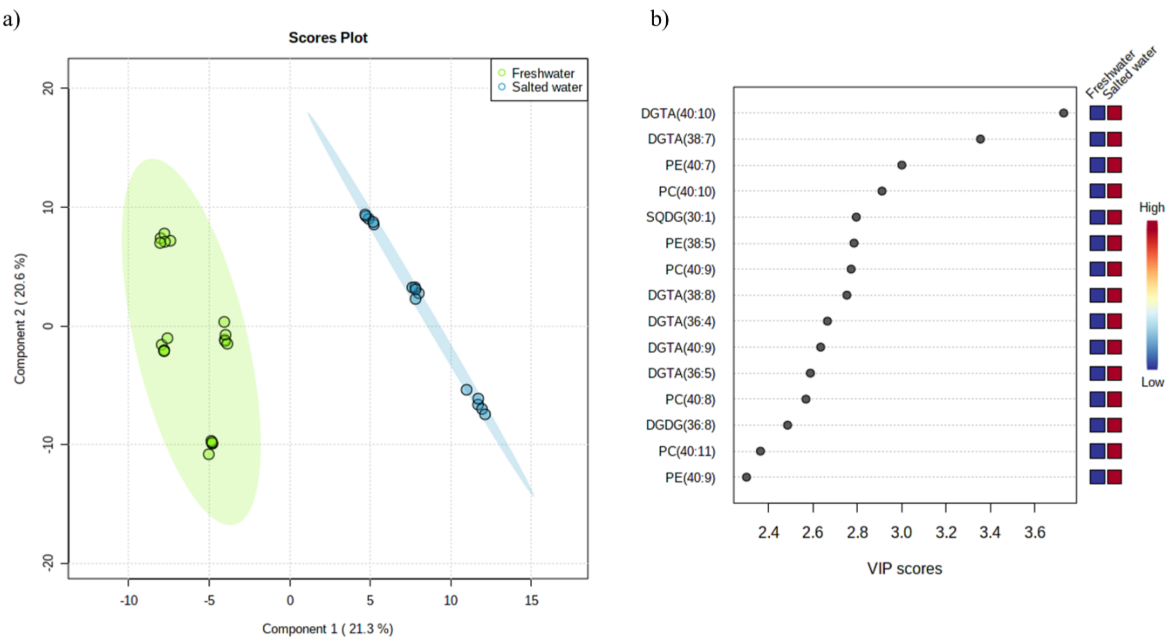


Figure 6.3. **a**) PLS-DA model of freshwater and saltwater species. **b**) VIP score.

We then selected the 50 most significant (lowest *p*-values) lipid species between the lipidome of marine and freshwater microalgae and plotted a clustering heatmap (**Figure 6.4**). The dendrogram at the top clearly shows the clustering of freshwater and saltwater microalgae. Fifteen lipid species were upregulated in freshwater microalgae, including 7 BLs (5 MGTS, 2 DGTS) 5 GLs (1 SQDG, 2 DGDG, 1 MGDG, 1 MGMG), and 3 PLs (2 PG, 1 LPG). Interestingly, marine microalgae have been characterized by up-regulation of lipid species with PUFAs, especially omega- 3 EPA. A cluster comprising 20 lipids (6 PC; 6 PE; 1 LPE; 1 DGTA; 2 DGDG; 4 MGDG) upregulated in marine microalgae was present. Except for *N. oceanica*, marine microalgae were characterized by up-regulation of BLs (DGTA and MGTA) lipid species.

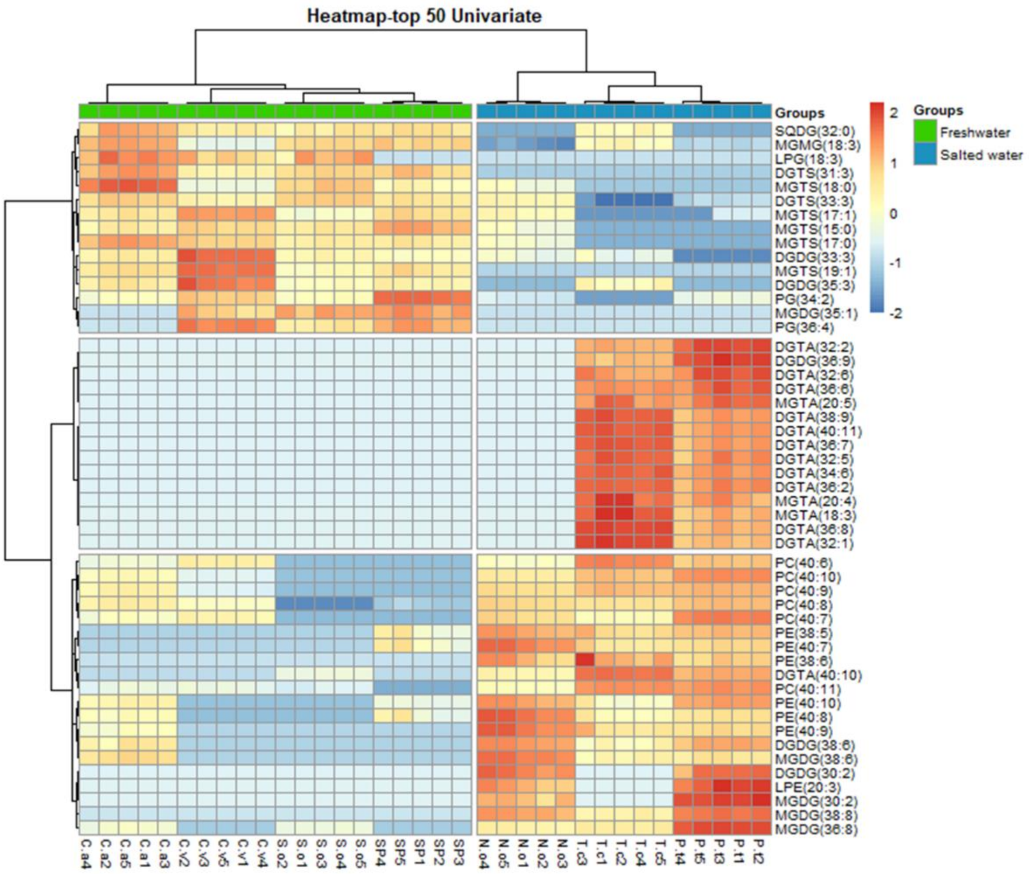


Figure 6.4. Two-dimensional hierarchical clustering heatmap of the 50 most significantly different polar lipids between saltwater (*T. chuii*, *N. oceanica* and *P. tricornutum*) and freshwater *C. vulgaris*, *S. obliquus*, *C. amblystomatis*, and *Spirulina* sp.) microalgae. The colour scale shows relative abundance levels, and the numbers indicate the fold difference from the mean. The dendrogram at the top represents the clustering of sample groups, showing two main clusters. The dendrogram on the left represents the clustering of individual lipid species given their similarity in relative abundance. Abbreviations: T.c, *Tetraselmis chuii*; S.o, *Scenedesmus obliquus*; C.a, *Chlorococcum amblystomatis*; C.v, *Chlorella vulgaris*; N.o, *Nannochloropsis oceanica*; P.t, *Phaeodactylum tricornutum*; SP, *Spirulina* sp.

A prediction model based on PLS-DA was also developed to classify microalgae species according to phylum from lipid data (**Figure 6.5**). Clustering between classes was obtained with the first two components with an accuracy=1, R^2=0.97, Q^2=0.96 (**Figure 6.5**). The most important variables in the PLS-DA model included 9 chloroplast-abundant lipid species (6 glycolipids and 3 PG), 4 PE and 2 betaine species.

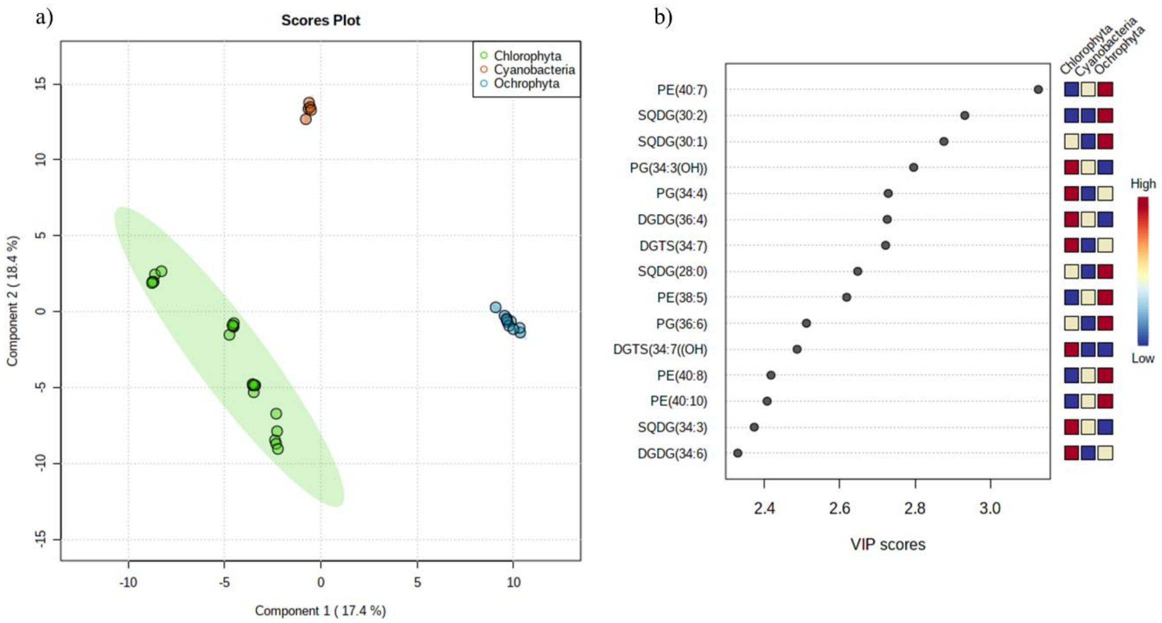


Figure 6.5. **a**) PLS-DA model of Chlorophyta, Ochrophyta, and Cyanobacteria species. **b**) VIP score.

As before, we selected the 50 most significant (lowest *p*-values) lipid species between the lipidome of the different phyla and plotted a clustering heatmap (**Figure 6.6**). The dendrogram at the top clearly shows the clustering of microalgae by phylum. Five lipid species were upregulated in Ochrophyta species, including 2 MGDG, 1 DGDG, 1 LPC and 1 LPE. Twenty-eight lipid species [25 PLs (19 PC, 6 LPC) and 3 GLs (3 DGDG)] were down-regulated in Cyanobacteria and most of them were up-regulated in Ochrophyta. Chlorophyta microalgae were characterized by up-regulation of chloroplast lipids, including 5 PG, 4 DGDG, 2 SQDG and 1 MGMG. Similarly, for the Chlorophyta microalgae, four chloroplast-abundant lipids [2 MGDG, 1 SQMG, 1 LPG] were upregulated in Cyanobacteria species.

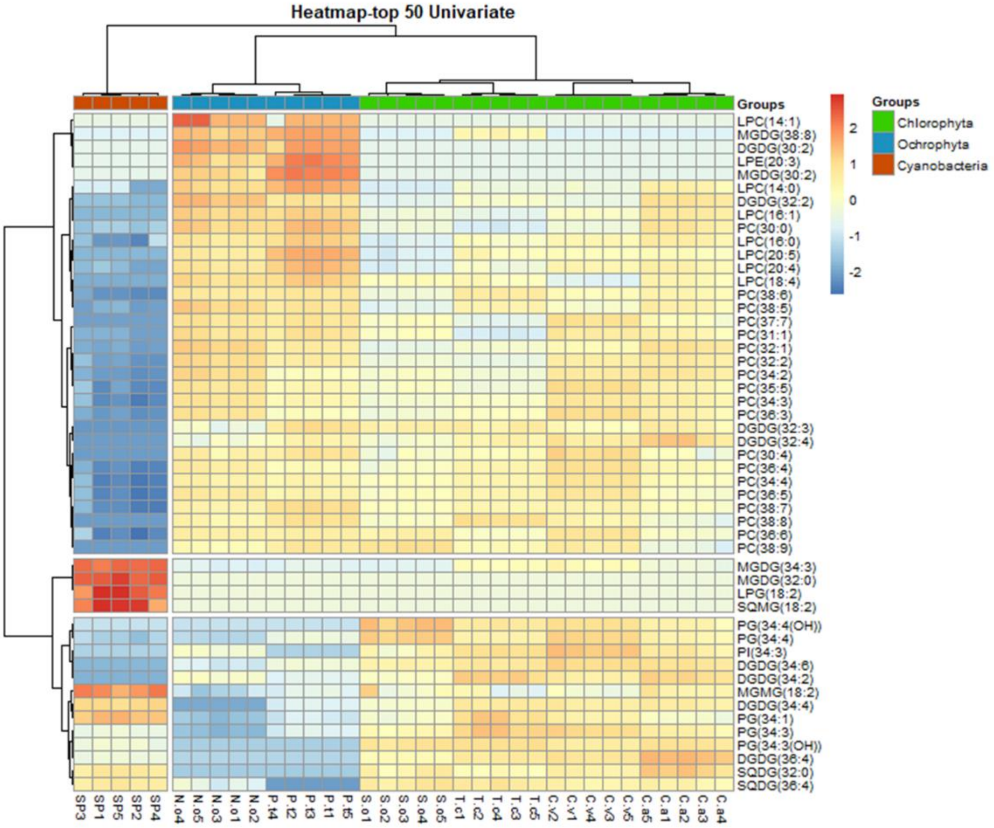


Figure 6.6. Two-dimensional hierarchical clustering heatmap of the 50 most significantly different polar lipids between phyla. The relative abundance levels are shown on the colour scale, and the numbers indicate the fold difference from the mean. The dendrogram at the top represents the clustering of sample groups, showing three main clusters. The dendrogram on the left represents the clustering of individual lipid species given their similarity in relative abundance. Abbreviations: T.c, *Tetraselmis chuii*; S.o, *Scenedesmus obliquus*; C.a, *Chlorococcum amblystomatis*; C.v, *Chlorella vulgaris*; N.o, *Nannochloropsis oceanica*; P.t, *Phaeodactylum tricornutum*; SP, *Spirulina* sp.

Finally, we performed a comparison between the lipid classes of the microalgae, based on the calculation of the sums of the normalized peak areas of the lipid species in each lipid class, to have a representation of the contribution of each class to the total lipid signature.

Regarding each lipid class, the most abundant lipid class within the GL group was SQDG in *Spirulina* sp., *P. tricornutum*, *C. amblystomatis* and *N. oceanica* or MGDG in *C. vulgaris*, *T. chuii* and *S. obliquus* (**Figure 6.7 and Supplementary Table S6.7**). The most abundant PL class was PG in *C. vulgaris*, *C. amblystomatis, S. obliquus*, *Spirulina* sp. and *T. chuii* and the second most abundant in *P. tricornutum* and *N. oceanica,* in which PC was the most abundant. In the BL group, DGTS was the most abundant class in *C. amblystomatis*, *S.*

obliquus and *N. oceanica*, with residual abundance in *Spirulina* sp., *C. vulgaris*, *P. tricornutum* and *T. chuii*. The DGTA class was most abundant in *T. chuii* and *P. tricornutum*, with a residual abundance in *N. oceanica* and *S. obliquus*.

Univariate analysis of the lipid class dataset (**Supplementary Table S6.8 and Table S6.9**) was performed, and the results showed significant changes in the 19 identified lipid classes (**Figure 6.7)** allowing robust discrimination between microalgae. For example, *T. chuii* had the highest amount of MGTA and DGTA, and the lowest levels of DGTS and MGTS, while *C. amblystomatis* had the highest cumulative levels of neutral GL, DGMG, MGMG, and DGDG. Acidic GL classes, such as SQDG and SQMG, were observed at the highest levels in *Spirulina* sp. and the lowest in *C. vulgaris*. On the other hand, the lowest amount of PL classes was observed in *Spirulina* sp., while *C. vulgaris* had the highest amounts of PC, PE and LPE classes.

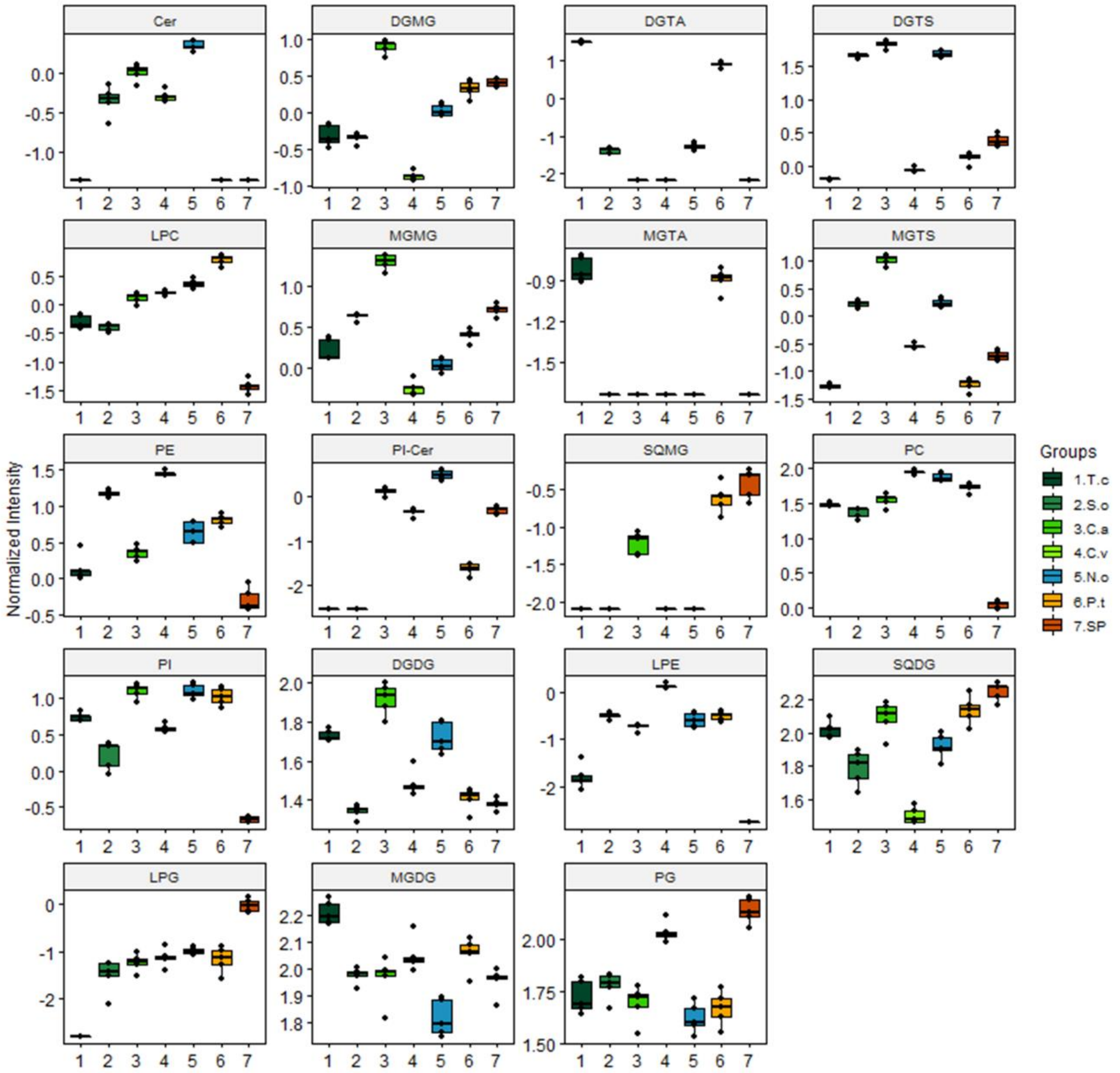


Figure 6.7. Boxplots of all identified polar lipid classes in microalgae. *Tetraselmis chuii* (T.c), *Scenedesmus obliquus* (S.o), *Chlorococcum amblystomatis* (C.a), *Chlorella vulgaris* (C.v), *Nannochloropsis oceanica* (N.o), *Phaeodactylum tricornutum* (P.t) and *Spirulina* sp. (SP). Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide.

The distribution of the sum of the classes of the same category, BL, GL, PL and SL, was plotted and is shown in **Figure 6.8**. All microalgae had high amounts of GL, followed by PL and BL, except *C. vulgaris*, which showed an opposite trend of GL and PL levels (**Figure 6.8**). *C. amblystomatis* exhibited the highest amount of GL followed by *T. chuii*, *Spirulina* sp., *P. tricornutum*, *N. oceanica*, *S. obliquus* and *C. vulgaris*. *C. amblystomatis* also had the highest BL content, followed by *N. oceanica*, *S. obliquus*, *T. chuii*, *P. tricornutum*, *Spirulina* sp., and *C. vulgaris*. SL lipids were absent in *T. chuii*, being more expressed in *N. oceanica*.

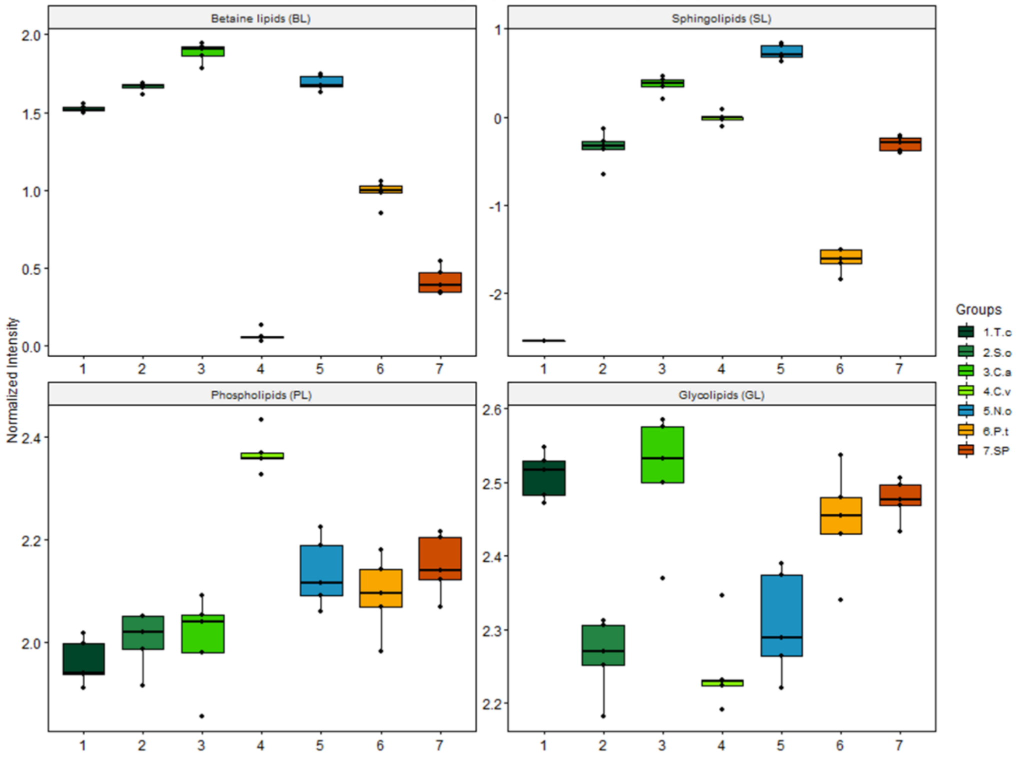


Figure 6.8. Box plots of the amount of each group of polar lipids (betaine lipids (BL), sphingolipids (SL), phospholipids (PL) and glycolipids (GL)) identified in the total lipid extracts of *Tetraselmis chuii* (T.c), *Scenedesmus obliquus* (S.o), *Chlorococcum amblystomatis* (C.a), *Chlorella vulgaris* (C.v), *Nannochloropsis oceanica* (N.o), *Phaeodactylum tricornutum* (P.t) and *Spirulina* sp. (SP).

4. Discussion

Microalgae have a complex and diverse biochemical composition, with several nutrients and phytochemicals that are of interest for biotechnological applications and may be used for chemotaxonomic classification. Among their highly diverse value-added and taxa-dependent compounds, FAs [13–17] and pigments [38,39] are widely investigated. But whether the polar lipid composition of the membrane enables chemotaxonomic differentiation and whether it can be useful for different applications and products remains unexplored. This is due to the fact that only a few microalgae have their lipidome identified and, among these studies, several were carried out using distinct methodologies, making it difficult to compare the specific lipid signature of each microalga or microalgae taxa.

The polar lipid signature of *Phaeodactylum tricornutum* [40–42], *Nannochloropsis oceanica* [37,43,44], *Chlorella vulgaris* [24,45], *Chlorococcum amblystomatis* [36], and *Scenedesmus obliquus* [46], as well as the photosynthetic glycerolipids of *Tetraselmis chuii* [47] have already been reported. Regarding *Spirulina* species, only the polar lipidome of *Spirulina platensis* [48] has been reported in the literature. In this study, we characterized and compared the polar lipidome of seven species of freshwater and marine microalgae belonging to three different phyla (Chlorophyta, Ochrophyta, Cyanobacteria): *C. vulgaris*, *C. amblystomatis*, and *N. oceanica*, *T. chuii*, *S. obliquus*, *P. tricornutum*, and *Spirulina* sp. These results were obtained using the same experimental approaches based on modern high-resolution LC-MS/MS lipidomic approaches thus allowing the comparative assessment of the specificity of polar lipids in different species.

The lipids identified in the seven microalgae were classified into four major lipid groups: PLs, GLs, BLs, and SLs (**Table 6.1**). Considering the previous work on the characterization of the polar lipidome of these microalgae [24,36,37,49], we have identified new classes and species of lipids in all microalgae. LPC and Cer were detected in *C. amblystomatis*, DGTA in *N. oceanica* and DGTS, MGTS and LPG in *C. vulgaris*. Although we did not report DGTS in *C. vulgaris* in our previous work [24], this class of lipids has already been found in *Chlorella* sp. [42,50]. Interestingly, Cañavate and colleagues suggested that this BL class could be a marker for green lineage microalgae [20]. DGTA is also reported for the first time in *S. obliquus*, *T. chuii* and *N. oceanica*. This BL class has already been reported in other species of Chlorophyta (*Tetraselmis suecica* [51]) and Ochrophyta (*N. gaditana* [51]). The presence of BL (DGTA and DGTS) in *S. obliquus*, *T. chuii*, *N. oceanica* and *P. tricornutum* corroborates that these microalgae can produce DGTA, since DGTS acts as the precursor of the polar group of DGTA [52].

Concerning *T. chuii*, only photosynthetic glycerolipids (MGDG, DGDG, SQDG and PG) have been previously identified [47]. In this work, besides DGTA, other lipid classes were also detected in *T. chuii*, namely PC and PE. Although PC and PE have not previously been reported in *T. chuii* [47], they have been identified in *Tetraselmis* sp. (*Tetraselmis* sp. Z3 and *Tetraselmis* sp. C6) [53]. PC and PE species have also been identified in the polar lipidome of *Spirulina* sp. This was the first time that these classes, along with LPC, DGTS, MGTS, and SQMG, were reported in this microalga. However, these classes of lipids have already been identified in other species of Cyanobacteria, namely *Scytonema julianum* (PC) [54], and *Gloeothece* sp. (PC, LPC, DGTS, MGTS and SQMG) [55], but never in *Spirulina* sp. Regarding *S. obliquus*, several classes of lipids were also identified for the first time, such as DGDG, DGMG, MGMG, DGTS, MGTS, DGTA, LPE and Cer.

A total of 498 different lipid species were identified and quantified across all classes. The comparison of the polar lipid composition of all microalgae revealed only 109 common lipid species and several unique lipid species for each microalga, revealing the specific lipid signature of each microalga (**Table 6.2** and **Supplementary Table S6.1**). Common species were mainly cell membrane lipids and a few plastid lipids, including 55 PLs (26 PC, 6 LPC, 13 PE, 9 PG, 1 PI), 37 BLs (27 DGTS, 10 MGTS) and 17 GLs (5 DGDG, 2 DGMG, 3 MGDG, 2 MGMG, 5 SQDG) lipid species (**Table 6.3**).

Regarding all the lipid species identified, a higher number of PLs was observed compared to BLs and GLs (**Table 6.1**). This is in agreement with what has been reported for *Scenedesmus* sp. [45], *Scenedesmus obliquus* [46], *Chlorella* sp. [50,56], *Chlorella vulgaris* [24,45], and *N. oceanica* [37,43,44,49], but different from what has been reported for *Spirulina platensis* [48], *C. amblystomatis* [36] and *P. tricornutum* [40].

Within PLs, several classes of lipids have been identified. Consistent with published data, the PC class was the lipid class with the highest number of lipid species in all microalgae, followed by PE or PG, as reported for *N. oceanica* [37], *Scenedesmus* sp. [45], *C. amblystomatis* [36], *C. vulgaris* [24] and *P. tricornutum* [40,41]. The other classes were less numerous but composed of relatively abundant lipid species. Interestingly, the relative abundance of some PL classes was different between prokaryotic and eukaryotic microalgae.

Prokaryotic cells of *Spirulina* sp. were characterized by higher amounts of PG, LPG and lower amounts of PC, PE and LPC. These results are consistent with data previously reported for species of Cyanobacteria, considered the ancestors of chloroplasts, in which PG is the most abundant class of phospholipids in cell membranes [57]. Among eukaryotic microalgae, *C. vulgaris* was the richest in PLs, mainly PC, PE and PG. PLs are major constituents of cell membranes, especially PC and PE and a few are signalling molecules such as LPC, LPE and PI. PG is mainly located in the thylakoid membrane of chloroplasts [58]. PLs are attracting interest as food ingredients, as transporters of omega-3 and omega-6 PUFAs [59], and few have been reported with bioactive properties [60,61].

BLs are membrane lipids, but their function is not fully understood. Interestingly, we identified in all microalgae molecular species of DGTS and MGTS, and DGTA and its lyso form in two Chlorophyta and two Ochrophyta. At the phylum level, higher content of DGTS has been reported in microalgae belonging to the phyla Chlorophyta and Ochrophyta [42,51,62]. However, this trend is not verified for all species; for example, *P. tricornutum* (Ochrophyta) contains higher amounts of DGTA [40,51]. In this work, we also observed differences in the proportion of BL lipid classes in microalgae belonging to the same phylum: from the four microalgae belonging to the phylum Chlorophyta, three of them (*C. vulgaris*, *C. amblystomatis*, *S. obliquus*) showed higher DGTS content and absence or residual amounts of DGTA, while *T. chuii* had higher DGTA contents. Regarding the microalgae of the phylum Ochrophyta, we observed a higher content of DGTA compared to DGTS in *P. tricornutum*, while *N. oceanica* showed an opposite trend.

The total number of GL species showed an interesting correlation by phylum. Chlorophyta species had a higher number of DGDG, while in Ochrophyta species there was a higher number of MGDG species (**Table 6.1**). This is consistent with previous work that reported a higher number of DGDG *vs* MGDG species in Chlorophyta species (such as *Haematococcus pluvialis* [63], *Chlamydomonas nivalis* [64], *Chlorella* sp. [50,56] and *C. vulgaris* [24,65]), while a higher number of MGDG *vs* DGDG species was found in Ochrophyta species (*e.g.* in *P. tricornutum* [40–42], *N. Oceanica* [37], *N. gaditana* [40], *N. salina* [66], *Thalassiosira weissflogii* [66] and *Skeletonema costatum* [67]). A similar trend to Chlorophyta was observed in *Spirulina* sp. Although other works have reported a higher number of MGDG *vs* DGDG for the Cyanobacteria *Spirulina platensis* [48] and *Synechococcus elongatus* [68], similar results have been reported for *Gloeothece* sp. [55].

Among the studied microalgae, *C. amblystomatis* and *T. chuii* had the highest relative content of GLs.

Sphingolipids were detected in all microalgae except *T. chuii*. These lipids have been identified in other green microalgae of the phylum Chlorophyta, *e.g. Haematococcus pluvialis* [63], *C. vulgaris* [24] and *C. amblystomatis* [36] and in eukaryotic microalgae species of Ochrophyta [37,69,70], Myzozoa [69], and Haptophyta [69] as well as in prokaryotic microalgae of the phylum Cyanobacteria [71]. The SLs, PI-Cer(34:0) and PI-Cer(36:0), which we detect in *Spirulina* sp. have also been reported by Calvano *et al* [71]. Sphingolipids from microalgae are less reported than PLs, GLs and BLs because they are generally found in low abundance. From a chemotaxonomic point of view, authors have already suggested using SL lipids to support alga chemotaxonomy [69]. For example, in seaweeds, PI-Cer lipids appear to be phylum-specific as they are mainly reported in Rhodophyta species [19,72]. They have also been described in microalgae from three different phyla (Ochrophyta, Myzozoa and Haptophyta) [69]. Among the seven microalgae described in this study, we did not observe any sphingolipid trend by phylum.

Multivariate statistical analyses were carried out to have a more precise overview of the contribution of each lipid species by taking into account their identity and their relative abundance. The PCA in **Figure 6.1** showed a clear separation between microalgae, with no trend by phylum, suggesting a species-specific lipid signature. It is important to emphasize the greater distance in the second dimension between *Spirulina* sp. (prokaryote) and other microalgae (eukaryote), which is consistent with previous reports on their FA profiles [14].

The polar lipid profile was able to distinguish between freshwater and marine microalgae using PLS-DA (**Figure 6.3**) and cluster analysis (**Figure 6.4**). In marine microalgae, the predominant lipid species were DGTA lipids (**Figure 6.3b**) and polar lipids esterified with fatty acid with four or more double bonds, most likely the omega-3 PUFAs EPA (C20:5) and DHA (22:6) (**Figure 6.4**). Freshwater microalgae were richer in lipids esterified with PUFAs with three double bonds. Highly unsaturated FAs (EPA and DHA) are mainly associated with microalgae of marine origin [14,73,74], while ALA with three double bonds (C18:3), with freshwater microalgae [75] as well as terrestrial plants [76]. But in the literature, there are also reports of the presence of EPA and DHA in freshwater microalgae and a phylum trend. For example, freshwater microalgae like *Monodus subterraneus* [77] and *Trachydiscus minutus* [78], both Ochrophyta, can accumulate large amounts of EPA and

DHA. The FA content of diatoms and dinoflagellates from different habitats (marine, brackish and freshwater) were more related to the surrounding environment than the phylogeny, whereas the FA profiles seem to be explained by taxonomy [79]. In another study, which evaluated the published FA profiles of 208 species of phytoplankton (of marine and freshwater origin from six taxonomic groups), the authors suggested that the FA profile of phytoplankton is determined more by phylogeny than by growing conditions [80].

The polar lipid profile also distinguished microalgae by phylum using PLS-DA (**Figure 6.5)** and cluster analysis (**Figure 6.6**) models. Assessing the contribution of lipid species in the PLS-DA model (**Figure 6.5b**), it included 9 chloroplast lipid species (6 glycolipids and 3 PGs), 4 PEs and 2 betaine species. This shows that lipids abundant in the chloroplast are more discriminating for the phylum than lipids that are more abundant in extracellular membranes, such as PLs or BLs. This suggests that these chloroplast-abundant lipids have been further preserved in the evolution of microalgae belonging to the same phylum. It is known that the lipid composition of chloroplast membranes is highly conserved in microalgae. The structural characteristics of chloroplasts can be very different between groups of algae; some of them are characteristic of the phylum [81–83]. For example, the chloroplasts of microalgae belonging to the phylum Ochrophyta have one or two layers of periplastid membranes or chloroplast endoplasmic reticulum, which are not present in Chlorophyta or Cyanobacteria, as a result of multiple endosymbiosis [81–83]. These differences in the structural characteristics of the plastid membranes could justify a more phylum-specific GL and PG profile. The heatmap in **Figure 6.6** corroborated the importance of these lipids for phylum discrimination, revealing upregulation of chloroplast lipids, including 5 PGs, 4 DGDGs, 2 SQDGs and 1 MGMG in Chlorophyta, 2 MGDGs, 1 LPG and 1 SQMG in Cyanobacteria, and also 2 MGDGs and 1 DGDG in Ochrophyta. Moreover, downregulation of phospholipids, mainly PC and LPC, in Cyanobacteria compared to other microalgae was observed.

The sum of individual lipid species by lipid class (**Figure 6.7**) and lipid category (**Figure 6.8**) showed that *Spirulina* sp. had the highest levels of SQDG and the lowest levels of PC and PE, while *C. vulgaris* has the opposite. *T. chuii* displays the highest amount of DGTA and *C. amblystomatis* has the highest cumulative levels of neutral glycolipids (DGMG, MGMG, and DGDG). All microalgae had high amounts of GL, followed by PL and BL, except *C. vulgaris*. Among the microalgae, *C. amblystomatis* was the microalga with the highest GL and BL contents.

From a phylogenetic perspective, it has been considered that the fatty acid profile can be used to distinguish microalgae at the phylum level but not at lower taxonomic levels [13], indicating that FAs are not species-specific. This work allowed us to verify that the polar lipidome of each microalga is species-specific, making the polar lipid signature an important chemotaxonomic tool for lower taxonomic levels (such as species or strain level). Thus, the knowledge gathered from this study, together with the literature, suggests that FAs and polar lipid profiles could be a powerful tool in the chemotaxonomic classification of microalgae. However, the low number of microalgae species studied may be a limitation, and further studies are needed to unveil the potential of the polar lipid profile of microalgae as a chemotaxonomic tool.

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CHAPTER 7 - Bioprospecting the anti-inflammatory activity of lipid extracts and fractions of the *N. oceanica*

1. Introduction

Algae lipids have been associated with the ability to modulate the immune system [1]. The anti-inflammatory properties of lipid species, extracts and fractions have been described for some species of microalgae, namely the *Nannochloropsis* genus, such as *N. gaditana* [2,3], *N. granulata* [4] and *N. oculata* [5]. Choi *et al.* also reported the anti-inflammatory activity of the ethanolic extract of *N. Oceanica* [6], through their inhibitory effects of NF-κB in Raw 264.7 cells, and the inhibition of nitric oxide (NO) production and β-secretase activity in BV-2 cells. However, no work has been published reporting the anti-inflammatory effects of lipid-enriched fractions of *N. oceanica*. *N. oceanica* is rich in omega-3 EPA lipids [7–9], which have well-known anti-inflammatory effects [10]. Moreover, their FA and polar lipid profile contain high amounts of bioactive lipids with reported anti-inflammatory activities (such as PG(36:6) and DGTS(40:10)) highlighting *N. oceanica* as a promising source of anti-inflammatory lipids [9]. To fill this gap, we evaluated in this work the anti-inflammatory potential of the total lipid extract, as well as the DGDG/SQDG-, MGDG- and PL/DGTS- fractions of *N. oceanica*. The anti-inflammatory effects of the extracts were assessed by quantification of NO production and the expression of pro-inflammatory genes (*Il1b*, *Nos2*, *Ptgs2* and *Tnfα*) in RAW 264.7 macrophage cells stimulated with LPS and the NO scavenging activity.

2. Material and methods

2.1. Reagents

HPLC grade dichloromethane (DCM, CH_2Cl_2), absolute ethanol 96% (CH_3CH_2OH), and methanol (CH_3OH) were purchased from Fisher Scientific Ltd. (Loughborough, UK). The water was of Milli-Q purity (Synergy1, Millipore Corporation, Billerica, MA, USA). All other reagents were purchased from major commercial sources and were of the greatest purity available. Lipid internal standards were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA): 1,2-dimyristoyl-sn-glycero-3-phospho-(10-rac-glycerol) (dMPG), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (dMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphatidylinositol (dMPI), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (dMPE), 1,2-dimyristoyl-sn-glycero-3-phosphatidylserine (dMPS), 1-nonadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine (LPC), N-heptadecanoyl-D-erythro-sphingosine (Cer), and 1',3'-bis-[1-2-di-tetradecanoyl-sn-glycero-3-phospho]-sn-glycerol (CL).

2.2. Microalgae strain and culture media

Spray-dried *Nannochloropsis oceanica* biomass was provided by Allmicroalgae, Natural products S.A. located in Pataias, Portugal and cultivated as previously described [11]. Briefly, *Nannochloropsis oceanica* was cultivated under autotrophic conditions in Guillard's F2 culture medium and supplemented with a mixture of NaCl (Salexpor, Coimbra, Portugal) and magnesium (Necton, Olhão, Portugal) at 30 g/L salinity. The scale-up process of cultures, as well as the light and temperature growth conditions, were those described by Conde *et al.* [11]. Briefly, 5-L flask reactors were cultivated from 7 to 15 days, under continuous light exposition (700 μmol photons.m^2/s). Then, five 5-L flask reactors were used to inoculate one outdoor flat panel (FP) reactor (0.1 m^3 L), which was later sequentially scaled to 1 m^3 FPs.

2.3. Lipid extraction

Lipid extraction from *N. oceanica* with ethanol and ultrasounds bath (J. P Selecta S.A. Model 3000683, 2.6 L, internal dimensions: 9 × 23 × 13 cm, Barcelona, Spain) was performed as described previously [7]. Briefly, 25 mg of spray-dried biomass of *N. oceanica* was extracted four times using 2 mL of ethanol and 30 min of ultrasonication in an ultrasound bath (JP Selecta S.A. Model 3000683, Barcelona, Spain), followed by two minutes of vortexing and ten minutes of centrifugation (Selecta JP Mixtasel, Abrera, Barcelona, Spain) at 2000 rpm. The resulting supernatants were combined and dried under a stream of nitrogen.

For lipidomic analysis, the dried extracts were washed with 2 mL CH_2Cl_2, 1 mL CH_3OH and 0.75 mL Milli-Q water, followed by two minutes of vortexing and ten minutes of centrifugation at 2000 rpm. The organic layer was collected and dried under a stream of nitrogen and stored at −80 °C until further use.

2.4. Solid phase extraction (SPE)

Lipid extracts from *N. oceanica* were fractionated into DGDG/SQDG-, MGDG and PL/DGTS fractions using a modified methodology based on Pacetti's method [12]. Briefly, 10 mg of lipid extract from *N. oceanica* dissolved in 1500 µL of DCM was added to a glass column, containing 5 g of silica (Flash 40–60 µm - 60 Å, ACROS Organics, Geel, Belgium), followed by sequential elution with 30 mL of DCM (neutral lipid fraction), 45 mL of diethyl ether:acetic acid (98:2, v/v) (pigments fraction), 30 mL of diethyl ether: acid Acetic [98:2, v/v]:acetone:MeOH [9:1, v/v] (50:50, v/v) (MGDG fraction), 40 mL of acetone:MeOH (9:1, v/v) (DGDG/SQDG fraction) and 40 mL of MeOH (PL/DGTS fraction). All fractions were filtered with a total volume of 1.5 mL of DCM through a syringe (Hamilton Gastight Syringe, Model 1001, 1000 µL) using 0.45 µm pore size filters (Millex®-LH syringe driven filter unit, non-sterile). The fractions were then dried under an N_2 stream and stored at −20 °C until further use.

2.5. Quantification of phospholipids

The phospholipid content of the lipid extracts and fractions from *N. oceanica* was estimated based on Bartlett and Lewis protocol [13] and as detailed in [14]. Briefly, monosodium phosphate solution at 100 µg/mL was used for the preparation of phosphate standards of 0.1 to 2 µg of phosphorous. A volume of 125 µL of 70 % perchloric acid was used to dissolve the samples and standards followed by 60 minutes of incubation at 180 ºC in a heating block (Stuart, Staffordshire, UK). After that, a volume of 825 µL of milli-Q water, 125 µL of ascorbic acid 10% and 125 µL of 2.5% ammonium molybdate solution were added, followed by one minute of vortexing and ten minutes of incubation in a water bath at 100 °C. The absorbance of the samples was determined in an ultraviolet-visible (UV-Vis) spectrophotometer (Multiskan GO, Thermo Scientific, Hudson, NH, USA) at 797 nm.

2.6. Quantification of glycolipids

The glycolipid content of the lipid extracts and fractions from *N. oceanica* was estimated the orcinol colourimetric method, as detailed in [15]. Briefly, 2 mL of orcinol solution (0.2% in 70% H_2SO_4) was added to 40 µg of the dried lipid extracts (n = 3) and glucose standards at different concentrations. After incubation at 80 °C for 20 min, samples were cooled to room temperature. A calibration curve was prepared using glucose standards of 0 to 50 µg of glucose. The absorbance of the samples was determined in an ultraviolet-visible (UV-Vis) spectrophotometer (Multiskan GO, Thermo Scientific, Hudson, NH, USA) at 505 nm.

2.7. Liquid chromatography-mass spectrometry

Lipid extracts and fractions from *N. oceanica* were analysed by reverse phase liquid chromatography in a Dionex Ultimate 3000 (Thermo Fisher Scientific, Bremen, Germany) using an Ascentis® Express 90 Å C18 column (Sigma-Aldrich®, 2.1 x 150 mm, 2.7 µm) coupled to the Q-Exactive® hybrid quadrupole Orbitrap mass spectrometer (Thermo Fisher, Scientific, Bremen, Germany) as detailed Couto *et al*. [14]. For this analysis, 5 µl of the following stock solutions were injected: 40 µg of lipid extract and the PL/DGTS fraction (determined by gravimetry) and 40 µg of DGDG/SQDG- and the MGDG fraction (equivalent to 40 µg of glycolipid determined by colourimetric assay), all dissolved in 20 µL of dichloromethane, and 8 µL of internal standard mix and 72 µL of an isopropanol/methanol (50:50, v/v) solution. The mobile phases (A and B), the gradient of elution and the mass spectrometer conditions were as follows: 32% B at 0 min, 45% B at 1.5 min, 52% B at 4 min, 58% B at 5 min, 66% B at 8 min, 70% B at 11 min, 85% B at 14 min, 97% B at 18 min, 97% B at 25 min, 32% B at 25.01 min and 32% B at 33 min. The mass spectrometer was operated in positive (ESI 3.0 kV) and negative (ESI −2.7 kV) modes, in cycles of a full-scan mass spectrum and ten data-dependent MS/MS scans. The following MS operational parameters were used: capillary temperature 320 °C, sheath gas flow 35 U, resolution 70,000, automatic gain control (AGC) target 3 x 10^6, *m/z* range 300-1600, 2 micro scans, maximum injection time (IT) 100 ms. MS/MS spectra were obtained using the following operational parameters: resolution 17,500, AGC target $1x10^5$, 1 micro scan, maximum IT 100 ms, dynamic exclusion 30 s, intensity threshold - 8 x 10^4, normalized collision energy (CE) 20, 24 and 28 eV in the negative mode and 25, 30 eV in the positive mode. Xcalibur data system (V3.3, Thermo Fisher Scientific, Bremen, Germany) was used to perform the data acquisition.

Lipid species were identified using mass spectrometry-data independent analysis (MS-DIAL) v4.70 software and manual data analysis, and integrated with the MZmine v2.53 software, as described previously [14].

2.8. Preparation of liposomes

Liposomes of the total lipid extract and the fractions containing DGDG/SQDG, MGDG and PL/DGTS were prepared with Dulbecco’s Modified Eagle Medium (DMEM) at 4 mg/mL. The weight of the total lipid extract and the PL/DGTS fraction was measured by gravimetry. The weight of the DGDG/SQDG and MGDG fractions was estimated by the quantification of the glycolipid using a colourimetric assay, as described previously.

Briefly, to prepare the liposomes, 2 mg of each extract were dissolved in dichloromethane. The solution was transferred to Eppendorf tubes, dried under an N_2 stream and redissolved with 500 µL of DMEM. Then, the samples were homogenized by vortexing for 10 minutes, sonicated for 15 minutes, vortexing for another 5 minutes, and sonicated for another 15 minutes.

2.9. Cell culture

RAW 264.7, a mouse leukaemic monocyte macrophage cell line (ATCC TIB-71, American Tissue Culture Collection, Manassas, VA, USA) was cultured in supplemented DMEM medium as previously described [16]. The cells were cultured in cell culture flasks (75 cm^2 cell culture OrFlask, Orange Scientific) and maintained in an incubator (Binder CB 150 CO_2) at 37 ˚C and 5% CO_2. The cell density was maintained between 0.5-0.8 × 10^6 cells/mL and cultures were sub-cultured every 2-3 days. For each assay, cells were used between passages 28 and 34.

2.10. Cell viability

The effect of the total extract and fractions on cell viability was determined by the resazurin reduction assay, as previously described [17]. Briefly, 200 µL of cells in complete medium at a density of 0.25 × 10^6 cells/well were seeded in a 96-wells plate (cell culture OrPlate, Orange Scientific), and left to stabilize overnight in an incubator (Binder CB 150 CO_2) at 37 ˚C and 5% CO_2. Then, the cells were kept in the culture medium (control) or were incubated with the lipid extracts at different concentrations (10, 25, 50, 100, and 200 µg/mL in the culture medium) in duplicate and left to stabilize in the incubator for twenty-four hours. After 24 h of incubation, the medium was replaced with 200 µL of a 50 µM resazurin solution prepared with the culture medium. The plate was then placed in the incubator for 1.5 hours, after which its absorbance was measured at 570 and 600 nm. Three biological replicates were carried out in duplicate, and the results were expressed as a percentage of cell viability, relative to the control. The highest non-cytotoxic concentration selected for subsequent assays were: 100 µg mL^{-1} for the total lipid extract, 50 µg mL^{-1} for the PL/DGTS fraction, 25 µg mL^{-1} for the DGDG/SQDG fraction; and 10 µg mL^{-1} for the MGDG fraction.

2.11. Assessment of the impact of lipid extracts on LPS-induced nitric oxide production

The production of NO was determined using a colourimetric assay with the Griess reagent as previously described [18]. Briefly, 200 µL of cells in complete medium at a density of 0.25 × 10^6 cells/well were seeded in a 96-wells plate (cell culture OrPlate, Orange Scientific), and left to stabilize overnight in an incubator (Binder CB 150 CO_2) at 37 °C and 5% CO_2. Then, the cells were maintained in the culture medium (control) or were incubated with the liposomes of the total lipid extract (100 µg mL^{-1} in culture medium), PL/DGTS fraction (50 µg mL^{-1} in culture medium), DGDG/SQDG fraction (25 µg/mL in culture medium) and MGDG fraction (10 µg mL^{-1} in culture medium). After 1 h of incubation, LPS was added to the selected wells, to a final concentration of 100 ng/mL, and the plate was incubated for 24 h. After this time, the medium in the wells was collected and centrifuged at 2000 rpm for five minutes. The supernatants were collected and stored at -20 °C.

The Griess reagent was prepared in the dark by combining reagents A and B in the proportion 1:1 (per volume). Reagent A was the (N-(1-naphthyl) ethylenediamine, 0.1% in 47 Milli-Q water) and B was 1% sulphanilic acid ($C_6H_7NO_3S$) in 5% phosphoric acid (H_3PO_4)).

Standard solutions of sodium nitrite ($NaNO_2$) were prepared in Milli-Q water at different concentrations (0.25, 0.5, 0.625, 1, 1.25, 2.5, 5, 10, 20, 50 and 250 µmol/mL). 80 µL of the standard solutions and supernatants were added to a 96-wells plate. Then, 80 µL of the Griess reagent was added to each well and the plate was incubated for fifteen minutes, in the dark and absorbance was measured at 550 nm in an ultraviolet-visible (UV-Vis) spectrophotometer (Multiskan GO, Thermo Scientific, Hudson, NH, USA). The results were expressed as a nitrite concentration. All measurements were performed in quadruplicate.

2.12. Assessment of nitric oxide scavenging activity

S-nitroso-N-acetyl-D, L-penicillamine (SNAP) was used to evaluate the NO scavenging activity of lipid extracts and *N. oceanica* fractions as previously described [19]. Firstly, 200 µL of culture medium was added to six Eppendorf tubes. For the extract and fraction samples, a volume of 5 µL of the liposomes of the total lipid extract was added to a concentration of 100 µg mL^{-1}; 2.5 µL of liposomes from the PL/DGTS fraction to a concentration of 50 µg mL^{-1}; 1.25 µL of the liposomes of the DGDG/SQDG fraction to a concentration of 25 µg mL^{-1} and 0.5 µL of the liposomes of the MGDG fraction to a concentration of 10 µg mL^{-1}. Then a volume of 0.8 µL of a 100 mM SNAP solution (to a concentration of 400 µM) was added, including a SNAP control. A solution with the culture medium, without SNAP or lipid extracts, was also prepared (control sample). All Eppendorf tubes were vortexed for thirty seconds before transferring 80 µL of each to a 96-wells plate, in triplicate. The plate was incubated for four hours, at 37 ºC (Heraeus T 5042 EK). Nitrite levels were determined by the Griess method, as described above. All measurements were performed in triplicate.

2.13. Real-Time Polymerase Chain Reaction (qPCR)

2.13.1. Ribonucleic acid (RNA) extraction

Macrophages at a density of 1 × 10^6 cells/ml in 1 mL of medium were cultured in a 12-well plate (Thermo Scientific Nunc) and left to stabilize overnight. Then, the cells were maintained in the culture medium (control) or were incubated with the liposomes of the total lipid extract (100 µg mL^{-1} in culture medium), PL/DGTS fraction (50 µg mL^{-1} in culture medium), DGDG/SQDG fraction (25 µg/mL in culture medium) and MGDG fraction (10 µg mL^{-1} in culture medium). After 1 h of incubation, LPS was added to the wells (except for the control samples), at a concentration of 100 ng/mL, and incubated for 24 h. Then, the medium in the wells was removed and 0.5 mL of the TRIzol® reagent was added to each well. The plate was incubated for five minutes at room temperature and the macrophages were manually homogenized. Then, the content of each well was collected in individual Eppendorf tubes followed by the addition of 100 µL of chloroform and fifteen seconds of homogenization. The tubes were incubated for three minutes at room temperature and centrifuged at 12000 G for fifteen minutes.

Then, the upper phase with ribonucleic acid (RNA) was collected in new Eppendorf tubes, followed by the addition of 250 µL of isopropanol, and incubated for twenty minutes at -20 ºC. Samples were centrifuged for ten minutes at 12000 G. To wash the RNA pellets, the supernatant was removed, and 1 mL of ethanol (75 %) was added, followed by vortexing and centrifugation for five minutes at 7500 G. The ethanol was removed, and the Eppendorf tubes dried in the Biosafety cabinet at room temperature, for five minutes. After this time, 30 μL of a commercial RNA storage solution at 60 ºC was added to the RNA pellets. The amount of RNA was estimated by measuring the absorbance at 260 nm, and its contamination was determined by the absorbance ratios 260/280 and 260/230 in a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA).

2.13.2. Synthesis of complementary DNA (cDNA)

The complementary DNA (cDNA) synthesis was performed with the NYZ First-Strand cDNA Synthesis Kit (Item No MB125, NYZTECH) according to the manufacturer's instructions and as described by Sousa et al. [20]. A total of 1 μg of mRNA from each Eppendorf tube was added to RNase-free water (to 8 μL), followed by 2 μL of reverse transcriptase and 10 μL of 2x reaction buffer. A thermocycler (CFX Connect, Bio-Rad) was used to incubate the Eppendorf tubes. The tubes were incubated (at 25º C for 10 min, at 50 ºC for 30 min, at 85 ºC for 5 min and cooled to 4 ºC), followed by the addition of 1 μL of RNAse H, incubated (at 37º C for 20 min) and addition of 79 μL of RNAse free water. Samples were stored at -20 ºC.

2.13.3. Real-time polymerase chain reaction (RT-qPCR) analysis

The real-time polymerase chain reaction (RT-qPCR) analysis was performed with each reaction mixture in a 20 μL volume containing 7.5 μL 2x NZYSpeedy qPCR Green Master Mix (Nzytech, Lisboa, Portugal), 2.5 μL of cDNA (25 ng), 2 μL of PCR grade water and l.5 μL of each primer (350 nM). Samples were incubated at 95 ºC for 2 minutes. After the initial denaturation step, subsequent 40 PCR cycles were performed as follows: 95 ºC for 5 sec., 55 ºC for 10 sec, and 72 ºC for 10 sec. Reactions were performed on a Bio-Rad CFX connected with technical duplicates for each sample. CFX Manager software was used to obtain gene expression levels (using *Hprt1* as a reference gene). Beacon Designer software version 7.7 (Premier Biosoft International, Palo Alto, CA, USA) was used to design the primer sequences.

2.14. Statistical analysis

Shapiro Wilk normality test was used to verify the normality of data. One-way ANOVA analysis, followed by Dunnett's multiple comparison post-hoc test was used to compare treatments with controls. A *p*-value < 0.05 was considered a statistical indicator of significance. All graphics and statistical tests were performed using GraphPad (version 8.0.2).

3. Results and discussion

3.1. Quantification of phospholipids and glycolipids

The PL/DGTS, DGDG/SQDG and MGDG fractions were obtained from the total lipid extracts of *N. oceanica* using solid-phase extraction (SPE). The amount of phospholipids and glycolipids present in the total lipid extract and fractions were estimated by colourimetric tests and the results are shown in **Table 7.1**.

Table 7.1. The amount of phospholipids and glycolipids present in the total lipid extract and fractions (obtained after solid phase extraction) was determined using colourimetric assays. The values are expressed in µg (of PL or GL) estimated by colourimetric assays per mg (of lipid extract or fraction) measured by gravimetry. Data are means ± SD.

Samples	Phospholipid content (µg/mg lipid extract or fraction)	Glycolipid content (µg/mg lipid extract or fraction)
Total lipid extract	68.1 ± 0.5	325.8 ± 5.6
DGDG/SQDG fraction	-	791.0 ± 10.0
PL/DGTS fraction	112.8 ± 9.2	-
MGDG fraction	-	599.6 ± 41.4

These results are similar to those reported for lipid extracts of *N. oceanica* extracted with a dichloromethane/methanol solution [9]. In this study, 1 mg of *N. oceanica* lipid extract consisted of 104.8 µg PL and 235.2 µg GL when the cultures were grown outdoors and 75.3 µg PL and 235.2 or 274.8 µg GL when grown indoors. The results presented in **Table 7.1** enabled us to corroborate that, compared to the total lipid extract, the PL/DGTS fraction was richer in phospholipids (fraction=112.8 ± 9.2 µg/mg *vs* total lipid extract=68.1 ± 0.5 µg/mg), while the DGDG/SQDG and MGDG fractions (791.0 ± 10.0 and 599.6 ± 41.4 µg/mg, respectively) were richer in glycolipids than the total lipid extract (325.8 ± 5.6 µg/mg). Interestingly, the PL/DGTS fraction contained 112.8 µg per mg of fraction, suggesting that this fraction is either enriched in DGTS lipids or that this fraction contains a significant amount of impurities or unknown compounds. Only two lipidomic studies in *N. oceanica* reported the absolute quantification of the lipid classes of phospholipids, glycolipids and betaines [21,22], but obtained different results. While Han *et al*. reported that *N. oceanica* IMET1 had more PC and PG than DGTS [21], Menge *et al*. reported that under normal growth conditions, *N. oceanica* had a higher amount of DGTS (20.8%) than PC (10.3%) or the other PL classes (PE, PG, and PI, less than 5 %) [22].

Moreover, we can also observe that in the case of the MGDG fraction, only about 60 % of the fraction is composed of glycolipids. The remaining 40 % of the fraction, may be predominantly carotenoids (because this fraction had a slight brown colour). The DGDG/SQDG fraction appears to be the purest fraction with approximately 80 % of the fraction being composed of glycolipids, and only 20 % of unknown compounds.

3.2. Characterization of lipid extracts and polar lipid fractions of *N. oceanica* by LC-MS

The polar lipid compositions of total lipid extracts and fractions (DGDG/SQDG, MGDG, PL/DGTS) of *N. oceanica* were characterized by LC-MS (**Supplementary Figures S7.1-S7.6**) and MS/MS, as previously reported [8,9]. The chromatograms of each fraction and the retention times of the PL, GL and BL classes are shown in **Figure 7.1 and Figure 7.2**. The results showed that the polar lipid classes present in the total lipid extract were recovered after SPE fractionation in each corresponding fraction.

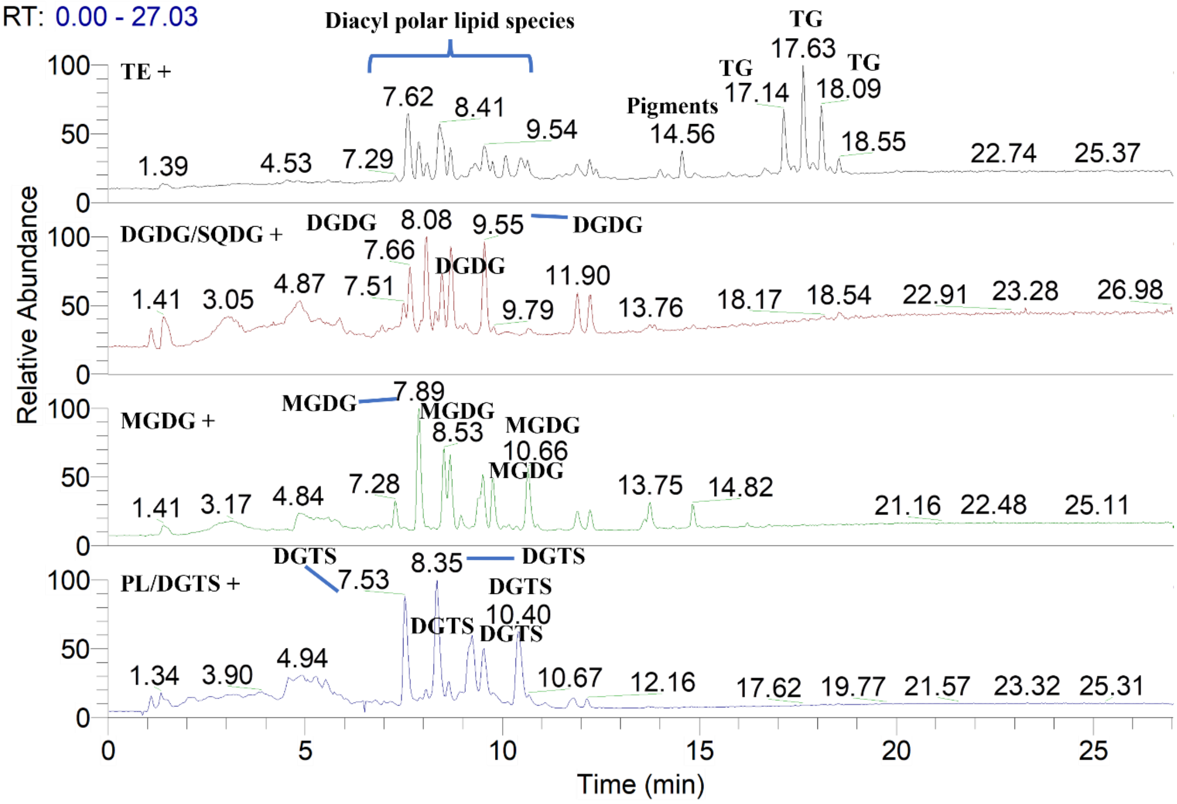

Figure 7.1. Total ion chromatogram of lipid extracts and fractions of the *N. oceanica* obtained in the positive (+) mode.

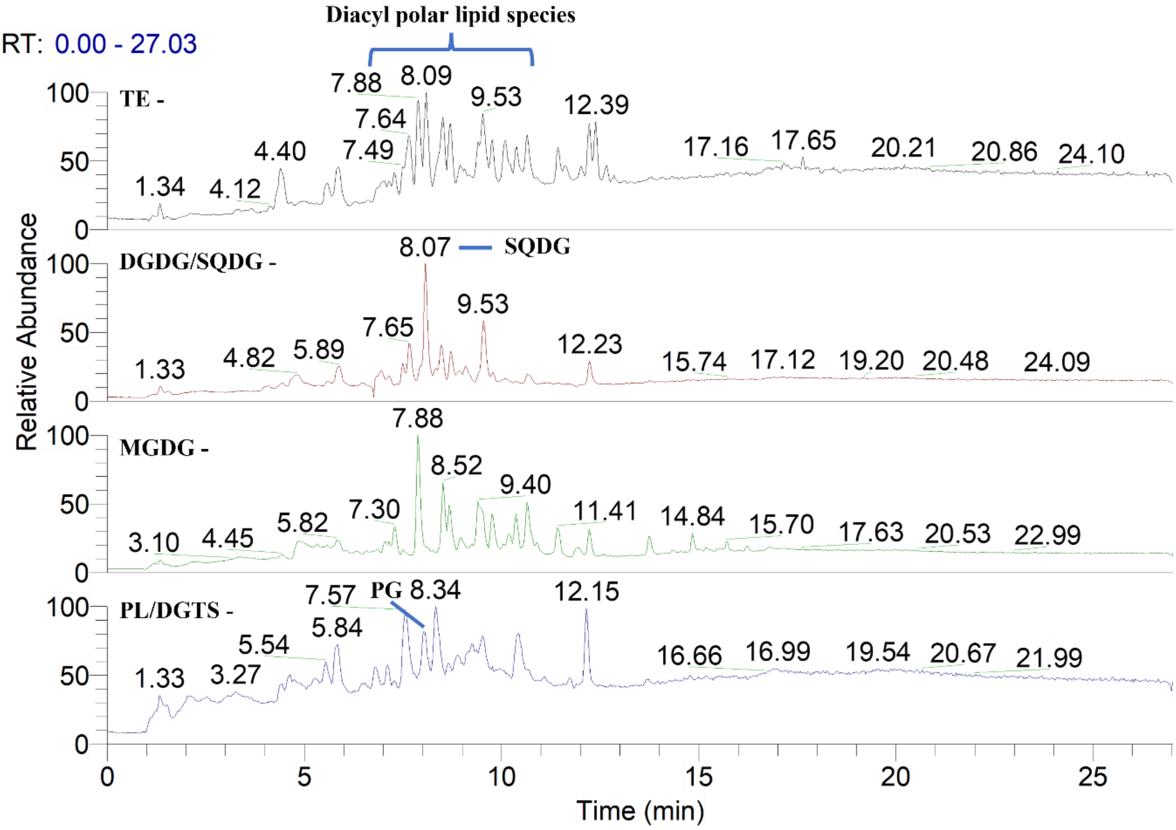

Figure 7.2. Total ion chromatogram of lipid extracts and fractions of the *N. oceanica* obtained in the negative (-) mode.

3.3. Effect of *N. oceanica* extracts and fractions on cell viability of RAW 264.7 macrophages

The viability of RAW 264.7 macrophages was evaluated by the resazurin assay. This assay was carried out to monitor the cytotoxicity of the extracts by measuring the number of viable cells (with active metabolism). Macrophages were treated with total lipid extracts and three fractions (PL/DGTS; DGDG/SQDG and MGDG) of *N. Oceanica* at five different concentrations (10, 25, 50, 100, 200 µg mL^{-1}) (**Figure 7.3**). All treatments induced 100% cell death at the highest concentration tested (200 µg mL^{-}), except the total lipid extract and PL/DGTS fraction. The highest concentrations of lipid extracts and fractions that did not compromise the cellular viability were: 100 µg mL^{-1} for the total lipid extract, 50 µg mL^{-1} for the PL/DGTS fraction, 25 µg mL^{-1} for the DGDG/SQDG fraction; and 10 µg mL^{-1} for the MGDG fraction. These concentrations were used in further assays to assess the ability of these extracts/fractions to inhibit the LPS-induced NO production and NO scavenging activity and to inhibit the LPS-induced transcription of pro-inflammatory mediators.

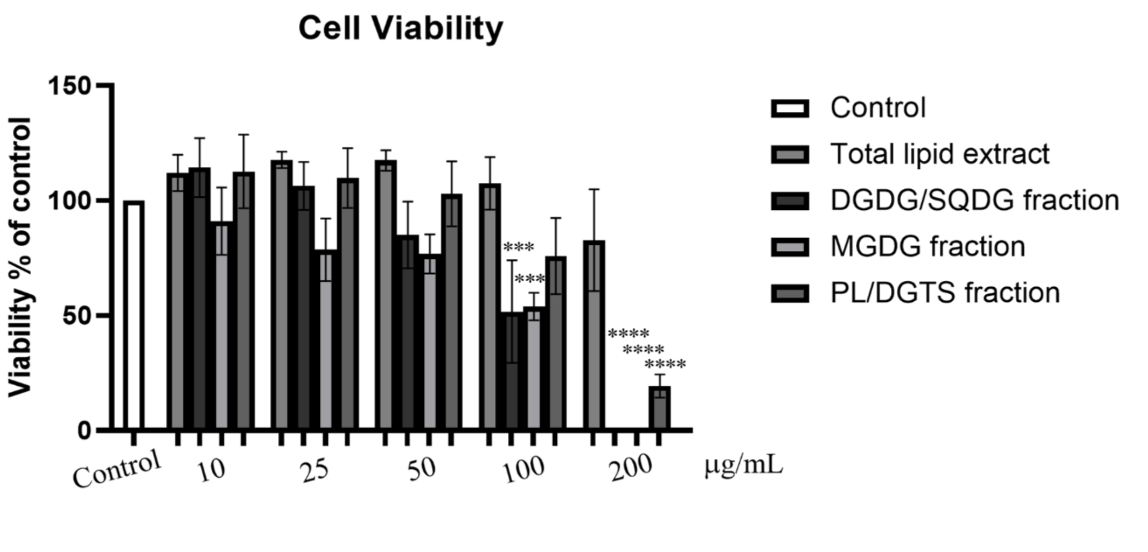

Figure 7.3. Effects of five different concentrations (10, 25, 50, 100, 200 µg/mL) of lipid extracts and fractions (DGDG/SQDG; PL/DGTS; MGDG) of *N. oceanica* on the viability of RAW 264.7 macrophages. The results are expressed as percentage reduction of resazurin relative to the control. Data are means ± SD, $n = 3$. *** $p < 0.001$ and **** $p < 0.0001$ (*vs* control) using one-way ANOVA independent-measures test followed by Dunnett's test).

Few studies have reported the effects of extracts and fractions of *N. oceanica* on the viability of the raw 264.7 macrophage cell line. Samarakoon *et al.* reported the effects of three fractions (hexane, chloroform and ethyl acetate fractions) obtained from methanolic extracts of *N. oceanica* in RAW 264.7 cells by MTT assay [23]. The hexane and the chloroform fraction were evaluated at different concentrations (6.25, 12.5, 25, and 50 µg/mL), but only the lower concentration fractions (6.25 µg/mL) did not affect RAW cell viability, while the others decreased cell viability [23]. Choi *et al*. assessed the ability of ethanol extracts of *N. Oceanica* (at 20 µg/mL) to inhibit NF-κB luciferase activity in Raw 264.7 macrophages, and to impact the cell viability of neuronal cells, and this extract concentration did not affect the cell viability [6].

Cytotoxic effects were also evaluated for lipid extracts from other *Nannochloropsis* species. The cytotoxicity effects of five fractions of methanolic extract of *N. oculata* were also determined by MTT assay on RAW 264.7 cells [5]. In this work, the authors reported that all the fractions tested at different concentrations (6.25 and 12.5 µg/mL) were not cytotoxic for the cells, and two of them were also not cytotoxic at a concentration of 25 µg/mL [5]. No cytotoxic effects were observed on THP-1 macrophages exposed to EPA-derived oxylipin from *N. gaditana* at different concentrations (25, 50, and 100 µM) [2]. The diacylglyceryltrimethylhomoserines (1–6) isolated from *N. granulata* at 25, 50, and 100 µM also had no cytotoxic effect on RAW264.7 macrophage [4].

3.4. Effect of *N. oceanica* extracts and fractions on LPS-induced NO production in RAW 264.7 macrophage cells

The effects of *N. oceanica* lipid extracts and fractions (PL/DGTS; DGDG/SQDG and MGDG) on NO production in LPS-stimulated (100 ng/mL) and un-stimulated RAW 264.7 macrophage cells were measured by colourimetric assay with Griess reagent (**Figure 7.4**).

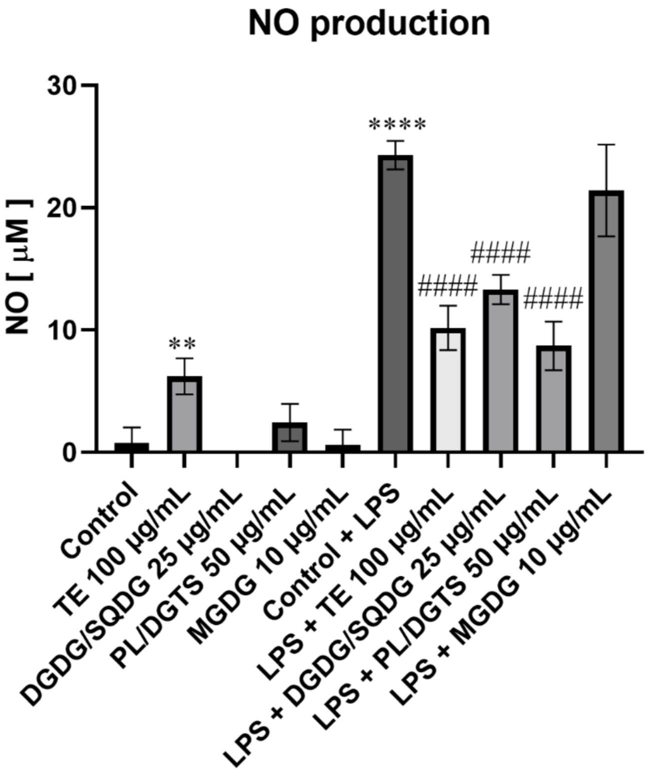

Figure 7.4. Nitric oxide (NO) production on un-stimulated and LPS-stimulated RAW 264.7 macrophage cells treated with lipid extracts (TE) and 3 fractions (PL/DGTS; DGDG/SQDG and MGDG) of *N. oceanica*. Results were expressed as nitrite concentration (µM) of four independent biological assays (*n*=4) ± SD. ** *p* <0.01 and **** *p* <0.0001 (*vs* control); #### *p* < 0.0001 (*vs* LPS) using one-way ANOVA independent-measures test followed by the Dunnett's test.

Treatment of macrophages with the extracts in the absence of LPS did not induce significant production of nitrites compared to the control, except for the total lipid extract (**Figure 7.4**), suggesting that these extracts do not promote an inflammatory macrophage response. Interestingly, co-incubation of LPS and each extract (total lipid extract, PL/DGTS and DGDG/SQDG fractions), induced a significant decrease in NO production from LPS-stimulated macrophages, showing a strong anti-inflammatory (immunomodulatory) potential. The MGDG fraction induced a slight but not significant reduction in nitrite levels.

The anti-inflammatory potential of lipid extracts and lipid fractions of *Nannochloropsis* spp. has been previously assessed by their inhibitory effect on NO levels in LPS-stimulated macrophages [4,24]. As is the case of six fractions enriched in individual DGTS obtained after fractionation and purification of an extract obtained with MeOH/CH_2Cl_2 of *N. granulate* [4]. Each fraction had as its major component one of the following DGTS: [DGTS(20:5/20:5), DGTS(20:5/20:4), (DGTS(20:5/14:0), DGTS(20:5/16:0), DGTS(20:5/16:1), DGTS(20:5/18:2)] [4]. Among them, betaine lipids carrying fatty acids with a higher degree of unsaturation [DGTS (20:5/20:5) and DGTS (20:5/20:4)], had higher inhibitory activity compared to others, showing the importance of the fatty acid composition of the lipid on their anti-inflammatory effect [4]. As previously reported, the total lipid extract and PL/DGTS fraction of *N. oceanica* are enriched in DGTS (20:5/20:5) and DGTS (20:5/20:4) species, which may explain the strong anti-inflammatory activity observed for the two extracts.

Our results show us that the DGDG/SQDG fraction of *N. oceanica* also significantly inhibits NO levels in LPS-stimulated macrophages. In *N. oceanica,* the most abundant DGDG species are DGDG(36:5) [assigned as DGDG(16:0-20:5) and DGDG(16:1-20:4)] and DGDG(36:6) [assigned as DGDG(16:1-20:5)] which have been previously reported with strong anti-inflammatory activity [25]. The presence of these DGDG species in the DGDG/SQDG fraction and the total lipid extract may explain the strong anti-inflammatory activity observed.

UFAs containing MGDG and DGDG were associated with anti-inflammatory activities. Fractions rich in MGDG and DGDG of *Isochrysis galbana* have been reported with anti-inflammatory activity determined through their ability to decrease TNFα levels in LPS-stimulated THP1 macrophages [24]. However, these fractions had lower activity than the individual DGDG (18:5/18:4) and DGDG (18:5/16:1) lipid species [24]. Pure DGDG and

MGDG species with the FA composition (20:5/16:0), (20:5/16:1), (20:5/14:0) and (20:5/20:5) of *N. granulata* have also been reported with anti-inflammatory activities determined through their ability to decrease NO levels in LPS-stimulated RAW 264.7 macrophages [25].

Although the MGDG fraction was composed of MGDG species with reported anti-inflammatory activity (*e.g.* MGDG(40:10) and MGDG(36:6)) [25], it was the fraction with the least activity, although it was also the fraction tested with the lower concentration. In addition, these results could also indicate that the structure-function relationship is also important for the bioactivities, and that probably DGDG and SQDG could have stronger inhibitory effects than MGDG species. It is plausible that other compounds present in the fraction could antagonize the effect.

3.5. Effect of *N. oceanica* extracts and fractions on NO scavenging activity

The effects of *N. oceanica* extracts and fractions on NO scavenging activity were assessed by colourimetric assay using an NO donor S-nitroso-N-acetyl-D, L-penicillamine (SNAP) and Griess reagent (**Figure 7.5**).

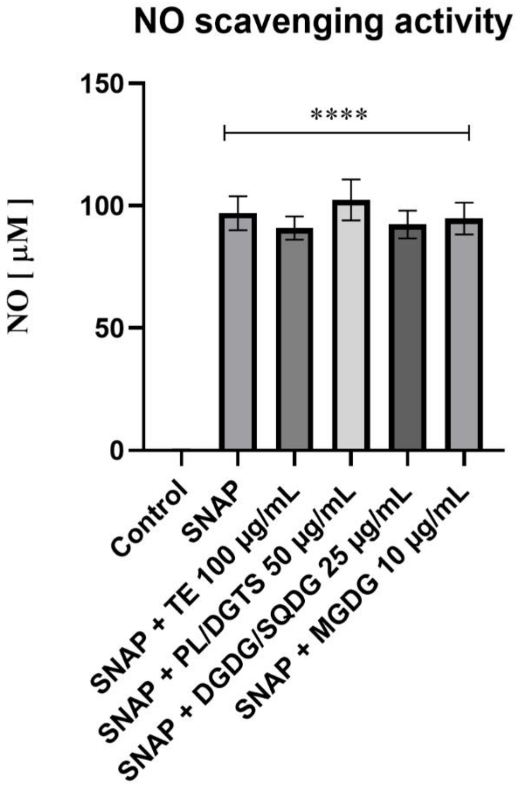

Figure 7.5. NO scavenging activity of lipid extract and fractions (DGDG/SQDG; PL/DGTS; MGDG) of *N. oceanica*. Results were expressed as nitrite concentration (µM) of three independent biological assays (*n*=3) ± SD. **** p <0.0001 (*vs* control) using one-way ANOVA independent-measures test followed by Dunnett's test.

The SNAP sample compared to the control induced a significant increase in NO levels (p < 0.0001). Co-incubation with extracts did not have significant effects on NO levels compared to the SNAP sample, suggesting that these extracts lacked NO scavenging activity and that their effects on inhibition of NO production by LPS-stimulated macrophages may result from downregulation of iNOS expression. No previous studies reporting the effects of lipid extracts or fractions of *N. oceanica* on NO scavenging activity have been published.

3.6. Effects of *N. oceanica* extracts and fractions on the transcription of pro-inflammatory mediators in LPS-stimulated RAW macrophages

The effects of *N. oceanica* extracts and fractions on the transcription of pro-inflammatory mediators in LPS-stimulated RAW macrophages were assessed using a quantitative real-time polymerase chain reaction (qPCR) (**Figure 7.6**).

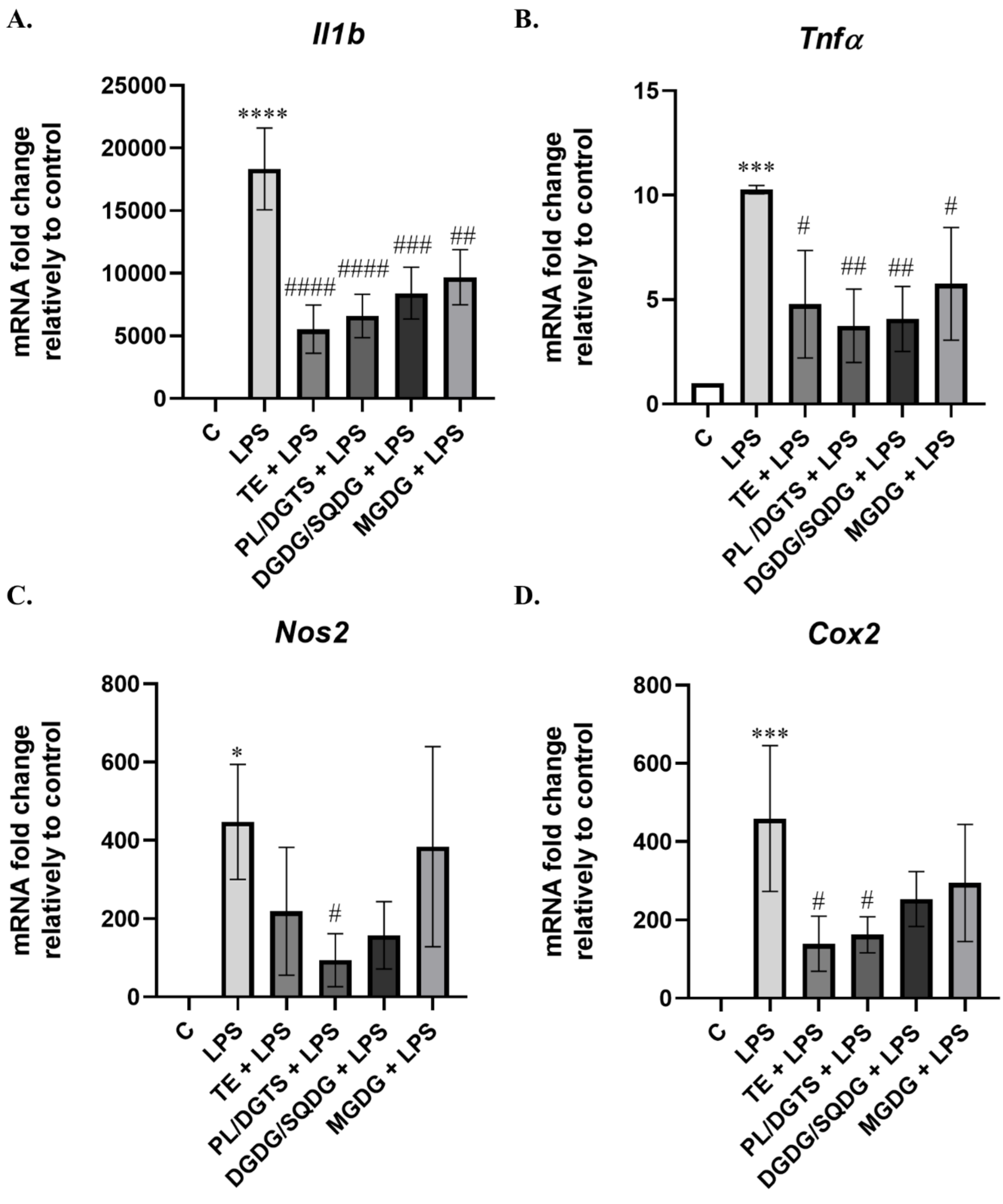

Figure 7.6. Effect of lipid extracts and fractions (DGDG/SQDG; PL/DGTS; MGDG) of *N. oceanica* on mRNA levels of genes (**A**) *Il1b*, (**B**) *Tnfa*, (**C**) *Nos2* and (**D**) *Cox2* in LPS-stimulated RAW 264.7 macrophages. Results were expressed as mRNA fold change relative to control from three independent biological assays (n=3) ± standard deviation. * $p < 0.05$ (*vs* control); ***$p < 0.001$ (*vs* control); **** $p < 0.0001$ (*vs* control); # $p < 0.05$ (*vs* LPS); ## $p < 0.01$ (*vs* LPS), ### $p < 0.001$ (*vs* LPS); #### $p < 0.0001$ (*vs* LPS) using one-way ANOVA independent-measures test followed by Dunnett's test.

As expected, LPS stimulation significantly increased mRNA levels of all pro-inflammatory genes assessed (*Il1b*, *Tnfα*, *Nos2* and *Cox2*). Interestingly, we were able to observe that co-treatment with *N. oceanica* extracts led to a significant decrease in mRNA levels of these pro-inflammatory genes, enhancing their anti-inflammatory potential. In addition, no up-regulation was detected, suggesting the absence of a pro-inflammatory effect induced by the lipid extracts.

Transcription of *Il1b* and *Tnfα* (which encode IL-1β and Tnfα, respectively) was downregulated by exposure to all extracts tested. Among them, total lipid extract and PL/DGTS fraction caused higher downregulation of *Il1b* expression, followed by DGDG/SQDG- and MGDG fractions. In the case of *Tnfα*, the PL/DGTS and DGDG/SQDG fractions appear to have the highest anti-inflammatory potential, followed by the total lipid extract and the MGDG fraction to the same extent. Regarding *Cox2* (which encodes COX-2*)*, the PL/DGTS fraction and the total lipid extract caused a significant reduction in the mRNA abundance. Further complementary studies, such as an *in chemico* COX-2 assay, should be done to corroborate the anti-inflammatory potential of these extracts. Regarding *Nos2* (which encodes iNOS), only the PL/DGTS fraction had a significant ($p < 0.05$) lowering effect on LPS-stimulated macrophages. This result, together with the results obtained in the previous assays (NO production and scavenging activity), indicate that, in the case of the PL/DGTS fraction, the inhibitory activity on NO levels may be due to the downregulation of iNOS, likely due to the downregulation effect on LPS-induced transcription of *Nos2*.

No previous studies have reported the effects of *N. oceanica* lipid extracts or fractions on the transcription of pro-inflammatory mediators in LPS-stimulated RAW macrophages. As reviewed by Conde *et al.* [1], the anti-inflammatory potential of lipids from microalgae by transcriptomic analysis has been scarcely explored. In conclusion, these results indicate that all fractions have the ability to modulate the LPS-induced inflammatory response, but each showed specific effects that suggest the presence of a structure-function relationship. The PL/DGTS fraction had a broader anti-inflammatory potential through its ability to decrease the expression of all the pro-inflammatory mediators tested (*Il1b*, *Tnfα*, *Nos2* and *Cox2*) and the concentration of nitrite on LPS-stimulated macrophages. DGDG has also shown interesting anti-inflammatory effects. These results show that the tested lipid extracts and fractions (DGDG/SQDG; PL/DGTS; MGDG) of *N. oceanica* contained bioactive lipids with anti-inflammatory activities, highlighting *N. oceanica* as a promising source of anti-inflammatory lipids.

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CHAPTER 8 - Concluding remarks and perspectives

Concluding remarks and perspectives

Microalgae have attracted considerable interest due to their applications in different fields, mainly for food, feed, and nutraceuticals, among others. Microalgae can also be easily cultivated in aquaculture and on an industrial scale under controlled and sustainable conditions, not competing with land with other crops. These unicellular organisms contain a wide range of nutritious and bioactive compounds such as complex lipids and PUFAs, which makes them extremely attractive for a multitude of applications in different fields. Given their unique properties and abilities, they have been called "superfoods", "foods of the future" and “nature’s ‘green gold” with the potential to help achieve the six UN 2030 Sustainable Development Goals (SDG), including SDG 2 (Zero Hunger), SDG 6 (Clean Water and Sanitation), SDG 7 (Affordable and Clean Energy), SDG 12 (Responsible Consumption and Production), SDG 14 (Life Below Water) and SDG 15 (Life on Land) [1]. Microalgae are sustainable resources for coping with climate change scenarios since they sequester carbon dioxide 10 to 50 times more efficiently than vascular plants [2] and convert it into biomass and oxygen (more than 50 % of the Earth's oxygen [3]). Thus, algae have been recommended by the Green deal as an appropriate and valuable resource for present and future generations.

Most of the microalgae are oleaginous organisms and can contain up to 80 % lipids, with a lipid composition abundant in bioactive lipids esterified in omega-3 and omega-6 PUFAs, such as EPA and DHA that are rarely found in terrestrial plants. The high lipid content and the valuable lipid composition of microalgae have attracted the growing interest of researchers and entrepreneurs for the development of novel and innovative products with high-added value based on microalgae and also as an alternative to fish oils as sources of healthy lipids. However, the lack of knowledge of the lipidome of most microalgae hinders the exploitation of these microalgae for the prospection of bioactive lipids.

Microalgae can adapt to extreme environments due to their metabolic flexibility. Lipids as major constituents of cell membranes and responsible for maintaining the structural integrity and fluidity of cell membranes are essential for these adaptations. Thus, manipulation of microalgae culture conditions can be used to increase lipid production as well as lipids of interest, which will result in more valuable biomass and extracts. Although changes in the lipid composition of microalgae under different growth conditions have already been reported in a few cases, their lipid plasticity is far from being fully understood.

The results collected during this PhD thesis project provide knowledge not only on the polar lipid signature of several species of microalgae which are mostly commercially available, including different strains of *Chlorella Vulgaris* and different species of *Nannochloropsis*. This PhD thesis project also shed light on the lipid remodelling in response to the growing conditions of some of these species of microalgae and the bioactivities (antioxidant and anti-inflammatory) of their lipid extracts. Novel extraction procedures using ethanol assisted with *ultrasounds* were successfully applied to obtain a bioactive lipid extract from *chlorella vulgaris* useful for food and nutraceutical applications.

In Chapter 2, the lipidome and FA profile of green *C. vulgaris* grown under autotrophic (C-Auto) and heterotrophic (C-Hetero) conditions were characterized. We identified in C-Hetero and C-Auto lipid extracts, respectively, 167 and 173 lipid species belonging to thirteen lipid classes. Four lipid classes identified in this work had not been previously reported in *C. vulgaris*, including DGMG, MGMG, Cer and PI-Cer. Autotrophic conditions lead to higher lipid yields and increased content of glycolipids and polar lipids esterified with omega-3 PUFAs (namely C18:3 *n*-3; ALA and C16:3 *n*-3). Otherwise, heterotrophic conditions lead to the production of lipid extracts rich in phospholipids and with a higher content of saturated FAs (C16:0) and omega-6 PUFAs (C18:2 *n*-6; LA). Regarding the bioactivities of their lipid extracts, both *C. vulgaris* exhibited high antioxidant activity and COX-2 inhibitory capacity, but without statistical differences between them.

C. vulgaris is one of the most widely used species of algae in the world and one of the few approved for food consumption. Lipid extracts obtained from *C. vulgaris* are rich in omega-3 alpha-linolenic acid (ALA) and omega-6 linoleic acid (LA) polyunsaturated fatty acids (PUFAs) which can be used as food ingredients and in food supplements. They may also be useful for targeted applications, for example in the case of vegan or vegetarian ingredients as an alternative to fish oil or to replace fat of animal origin. However, traditional solvents used for lipid extraction are not allowed in the food industry. Thus, to produce lipid extracts of *C. vulgaris* usable for food applications, we evaluated the efficiency of lipid extraction and the lipid composition of lipid extracts of *C. vulgaris*, cultured under autotrophic and heterotrophic conditions. We have tested ethanol extraction (EtOH, a solvent allowed in the food industry) and ethanol extraction assisted by an ultrasound probe (UAE, a sustainable and environmentally friendly technology) and compared to the conventional dichloromethane: methanol (2:1, v/v) (DCM) extraction method. In this work, presented in chapter 3, we have shown that ethanol extraction assisted by an ultrasound probe allows obtaining lipid extracts of *C. vulgaris* with a polar lipid composition and properties similar to the conventional method. As such, this method may represent an alternative method for obtaining extracts for food applications. Regarding the bioactivities of the extracts, we found that the antioxidant activity of DCM extracts was comparable to that of EtOH and UAE extracts, which boosts the utilization of both (EtOH and UAE extracts) for future applications in the nutraceutical and food industries.

Despite the health benefits of *C. vulgaris* biomass, some of its organoleptic properties including taste, flavour and colour, are not unanimously accepted by consumers, which makes it more difficult to market it for food purposes. Therefore, Allmicroalgae developed two chlorophyll-deficient mutants of *C. vulgaris* of C-Hetero (assigned as C-White and C-Honey) induced by chemical mutagenesis (using ethyl methane sulfonate) with organoleptic improvement (taste and flavour) and nutritional characteristics (high protein content and interesting lipid content). In Chapter 4, the lipid profile of these two new food strains of *C. vulgaris* was characterized, and it was observed that the two *C. vulgaris* have a similar lipid content of the order of 4.8 % for C-White and 5.0 % for C-Honey, but lower than C-Hetero and C Auto. Despite the lower lipid content, they still have a lipid composition rich in essential lipids and bioactive polar lipids with significant benefits for human health (*e.g.* SQDG(32:0) and MGDG(34:1)). They are composed of the same classes of lipids as well as some major lipids (by lipid class) as those described in C-Hetero. However, the differences observed in the relative abundance of the lipids identified in the two mutants, highlight a strain-specific lipid signature. The main differences were recorded in PE and DGDG species with omega-3 PUFAs and Cer species (augmented in C-Honey), and lipids with omega-6 PUFAs, MGDG and oxidized PEs (increased in C-White). Both *C. vulgaris* showed lower antioxidant and anti-inflammatory potential than previously reported for C-Hetero. C-Honey had the most promising antioxidant results and nutritional quality values for *n*-6/*n*-3 ratio, h/H ratio, AI, and TI, while C-White had the best PI value and anti-inflammatory activity. Lipidomic studies in this work highlighted these two new strains *of C. vulgaris* as a source of healthy lipids and nutritional characteristics that can be used in the food and nutraceutical industries.

Species belonging to the genus *Nannochloropsis* are among the most cultivated and studied microalgae, due to their high lipid content and abundance of eicosapentaenoic acid (EPA). *Nannochloropsis* species can be grown in outdoor or indoor conditions. These culture conditions differ in light and temperature parameters, which are known to induce changes in the lipid composition of microalgae. In Chapter 5, we describe the influence of growth conditions, outdoor and indoor, in the modulation of the polar lipidome of two species of *Nannochloropsis* (*N. Oceanica* and *N. Limnetica*). The lipid signature of *N. Limnetica* has been revealed here for the first time. The two species share the majority of lipid species (out of a total of 220) and differ mainly in the relative abundance of certain betaine lipids (increased in *N. Oceanica*) and lysolipids (increased in *N. limnetica*). Indoor growth induced lipid productivity in both species. Candidate lipid biomarkers from outdoor (PG(36:6) and indoor systems [MGDG(34:2), MGDG(34:1)] have been proposed. The two lipid extracts obtained from *Nannochloropsis* species in both conditions have interesting characteristics as they had a higher abundance of glycolipids and oleic acid (in indoor conditions) and, a higher relative abundance of phospholipids, betaine lipids and eicosapentaenoic acid (EPA) (in outdoor conditions). This study emphasizes the influence of species and culture conditions, on FAs and polar lipid profile of *Nannochloropsis* spp., revealing that indoor farming is a promising condition to obtain reproducible lipid extracts of high value.

The results reported in Chapters 2, 4 and 5 highlight a species-specific lipid fingerprint, which may be useful for chemotaxonomic differentiation. However, the same chapters also show the influence of culture conditions on the polar lipid signature of microalgae. Thus, in Chapter 6, we tested the potential of polar lipids to support the classification of microalgae at the species-level, and whether this can reflect the environmental conditions in which microalgae live. To achieve our objectives, we characterized and compared the polar lipidome of seven species of major commercial microalgae belonging to three different phyla, namely Chlorophyta *Chlorella vulgaris*, *Scenedesmus obliquus*, *Tetraselmis chuii*, *Chlorococcum amblystomatis*, Ochrophyta *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and Cyanobacteria *Spirulina* sp, including freshwater and marine microalgae and using the same experimental approaches for lipidomic analysis. LC-MS analysis allowed the identification of a total of 498 lipids, including 109 common to all microalgae. Polar lipid signatures were species-specific but also allowed classifying the microalgae species according to phylum as well as freshwater and saltwater species. Lipids abundant in chloroplasts were the main contributors to the discrimination of microalgae phyla, suggesting that these lipids were more preserved in the evolution of microalgae belonging to the same phylum. On the other hand, betaine lipids as well as lipids esterified with EPA were more abundant in marine microalgae and lipids with ALA more abundant in freshwater microalgae.

Among the microalgae surveyed, *N. oceanica* was the microalga with the highest lipid content and abundance of EPA. EPA, lipid species present in the lipidome of *N. oceanica* and extracts of *N. oceanica* have been reported with anti-inflammatory properties. However, there are no studies that have evaluated the potential anti-inflammatory of lipid fractions enriched in phospholipids/betaine lipids, glycolipids and lyso-lipids obtained from the ethanol extract of the *N. oceanica*. This knowledge increases the awareness of *N. oceanica* lipids and provides insights into their structure-function relationship. In Chapter 7, we describe the potential anti-inflammatory of total lipid extract and DGDG/SQDG-, MGDG- and PL/DGTS- fractions from *N. oceanica* by their ability to decrease the expression of pro-inflammatory mediators (*Il1b*, *Tnfα*, *Nos2* and *Cox2*) and nitrite concentration on LPS-stimulated macrophages. The total lipid extract and all fractions of *N. oceanica* showed anti-inflammatory potential, however, the PL/DGTS fraction stood out by its ability to decrease the expression of all pro-inflammatory mediators that were tested.

Overall, this thesis contributes to increasing our knowledge of eleven commercial microalgae, including four novel food strains of *C. vulgaris*, through the complete characterization of their lipid profile, the identification of different bioactive lipids and the bioprospecting of their lipid extracts which, promote the sustainable development of novel food products, as well as products for specific markets such as products for the elderly, children, vegetarians and vegans. Additionally, this thesis provides insight into the metabolic adaptation of *C. vulgaris*, *N. Oceanica* and *N. Limnetica* lipids which is essential for adjusting culture conditions for specific biotechnological applications. Finally, we also demonstrated the feasibility of a food-grade extraction method to extract polar lipids from *C. vulgaris* that can be used as ingredients to design novel functional foods.

Despite the advances in science described in this thesis, much future work remains to be done, including: i) Identify the polar and apolar lipidome of thousands of microalgae yet to be studied.; ii) Evaluate different growth conditions for the enrichment of microalgae biomasses in bioactive lipids with benefits for human health and of interest to different industries; iii) Perform proteomics analysis under the most promising growth conditions to gain new insights into lipid biosynthesis and understand the most expressed lipid metabolism pathways. iv) Create public databases with the polar and apolar lipidome signature of each microalga, as well as the bioactivities and potential toxicity of their lipid extracts; v) Pursue the bioprospecting studies of microalgae lipid extracts for in *chemico* determination of antioxidant and anti-inflammatory activity, but also antimicrobial, anti-obesity, antilipemic, anti-steatosis, among others. The most promising extracts should also be evaluated for their bioactivity *in vitro* on cell lines and *in vivo* in control organisms and with known pathologies in which these extracts are expected to have beneficial effects, such as delaying the development of inflammatory diseases or having a therapeutic effect on these diseases. Clarifying which lipid group(s) or molecule with the best bioactivity is essential to identify the structure-activity relationship and thus continue to perform the fractionation of the most promising extracts into phospholipids, glycolipids, and neutral lipids.

Other related research areas that need to be further explored include: i) assessment of the bioavailability and bioaccessibility of microalgae extracts during long-term ingestion in animals and humans. This will be important nutritionally and in predicting the number of bioactive lipids that will reach the site of action. ii) The evaluation of the differences in lipid composition of microalgae grown on wastewater or seawater in a circular economy approach. For example, it is essential to assess the feasibility of integrated multi-trophic aquaculture (IMTA) systems using microalgae and aquatic organisms that feed on them such as bivalve molluscs (clams and oysters), crustaceans (shrimp), and herbivorous fish [4]. Most IMTA studies have been performed with macroalgae, which had lower growth rates, and photosynthetic rates, and exhibited lower lipid content than microalgae [5]. Thus, as future works, it will be important to evaluate IMTA systems with the microalgae with the most interesting lipid profiles such as microalgae belonging to the genus *Pavlova*, *Nannochloropsis* and *Isochrysis* and to characterize the changes over time in the lipid profile of aquatic organisms who feed on them. iii) The identification of the microalgae lipidome can be used for chemotaxonomic classification of microalgae, to monitor or be used to refine their growth in fresh/saltwater systems for future applications and new markets. iv) One of the biggest challenges in commercializing microalgae products is also the lack of marketing and advertising campaigns for existing products. Therefore, work on the dynamization of microalgae products and their health benefits along with continued research work is of utmost importance in the future. All this work will certainly promote the development of new products and markets, improve the quality of life of populations, and help preserve the oceans and marine resources for future generations, in line with the goals of the 2030 Agenda, the blue bioeconomy and the European Green Deal.

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Supplementary material

Supplementary material of Chapter 2

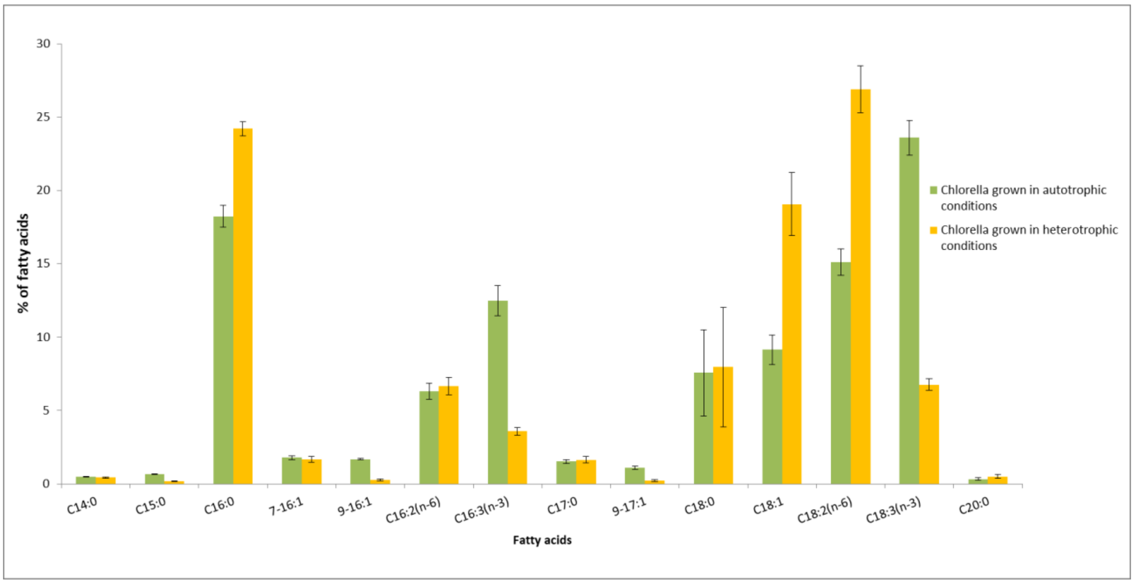


Figure S2.1. Fatty acid composition of total lipid extracts identified by GC-MS in *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions. Values are means ± standard deviation of five independent experiments.

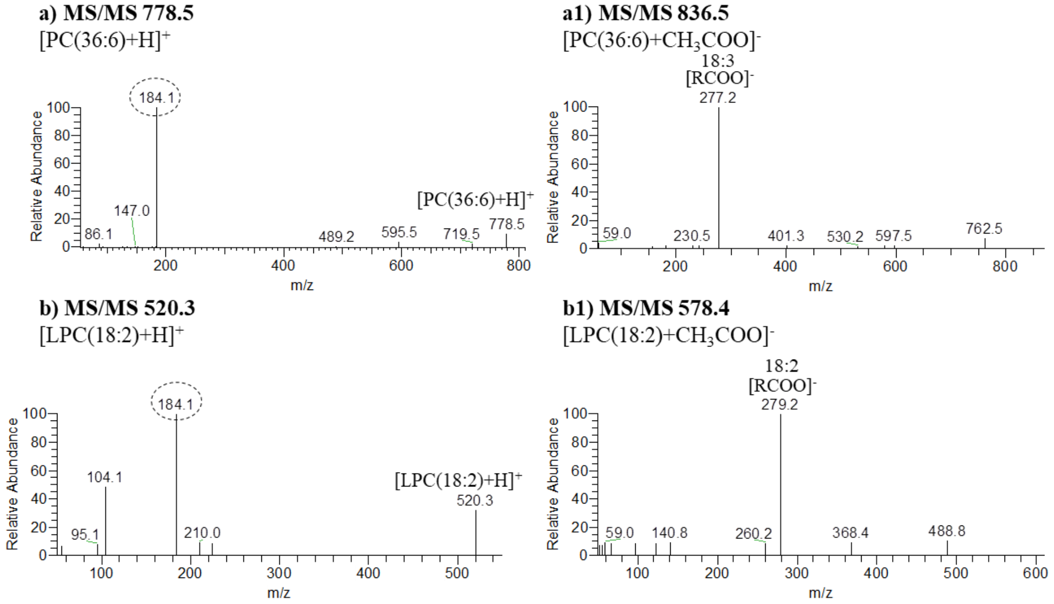


Figure S2.2. Example of HILIC-ESI–MS/MS spectra of $[M+H]^+$ molecular ions from phosphatidylcholine (PC) at *m/z* 778.5 (**a**) and lysophosphatidylcholine (LPC) at *m/z* 520.3 (**b**) with characteristic product ions corresponding to the phosphocholine polar head group at *m/z* 184 indicated by a grey circle. In negative mode, an example of HILIC-ESI–MS/MS spectra of $[M+CH_3COO]^-$ molecular ions from PC at *m/z* 836.5 (**a1**) and LPC at *m/z* 578.4 (**b1**) with the presence of the carboxylate $[RCOO]^-$ anions [48].

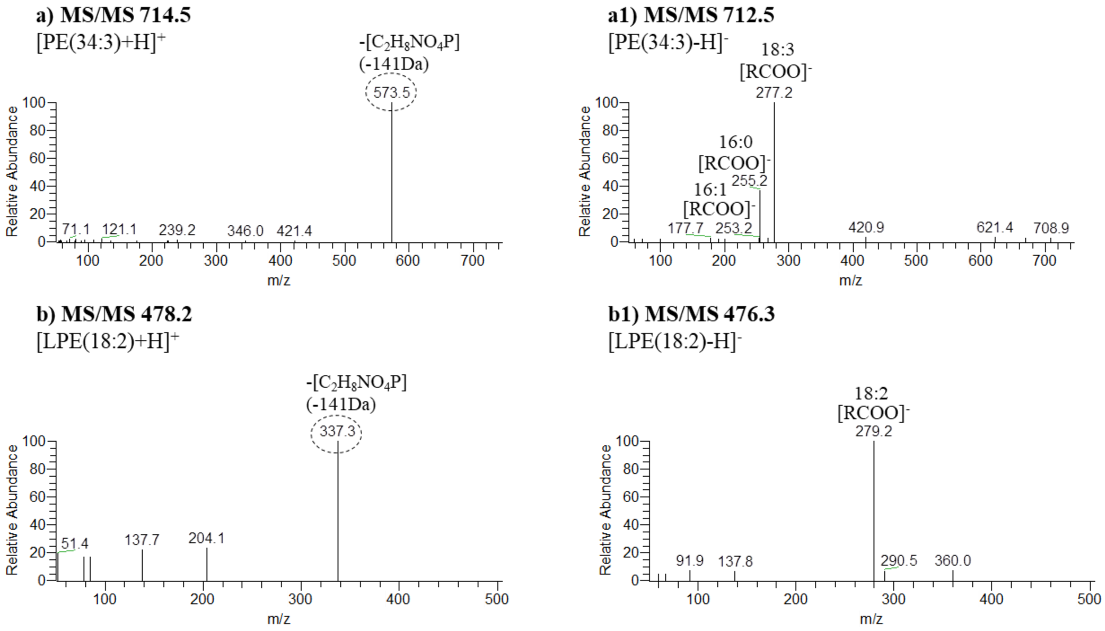


Figure S2.3. Example of HILIC-ESI–MS/MS spectra of $[M + H]^+$ molecular ions from phosphatidylethanolamine (PE) at *m/z* 714.5 (**a**) and lysophosphatidylethanolamine (LPE) at *m/z* 478.2 (**b**) with characteristic neutral loss of 141 Da corresponding to the loss of polar head group indicated by a grey circle. In negative mode, an example of HILIC-ESI–MS/MS spectra of $[M - H]^-$ ions of PE at *m/z* 712.5 (**a1**) and LPE at *m/z* 476.3 (**b1**) with a presence of the corresponding carboxylate $RCOO^-$ anions [48].

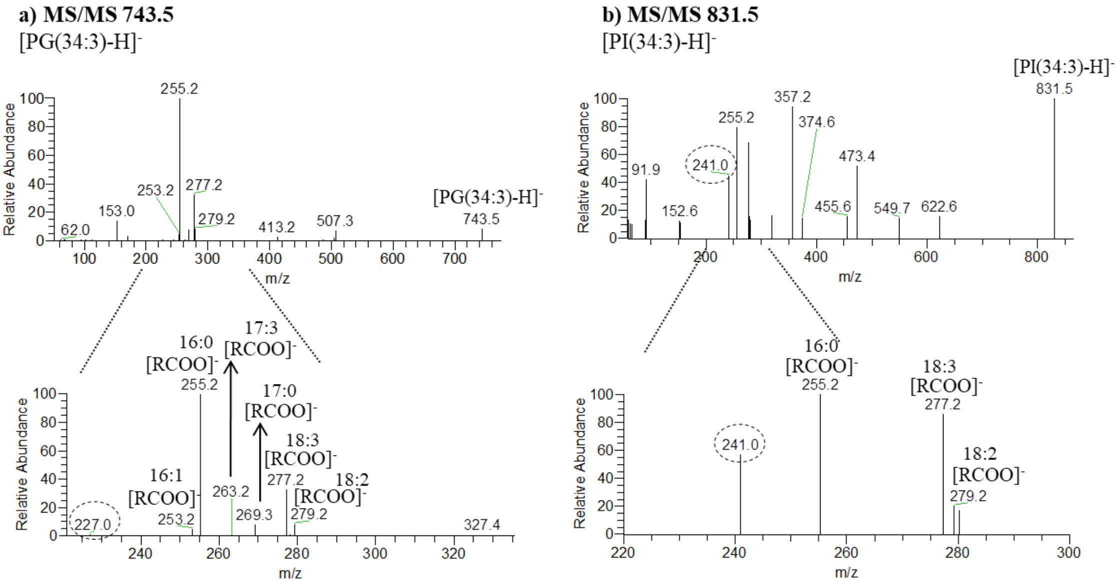


Figure S2.4. Example of HILIC-ESI–MS/MS spectra of the [M-H]$^-$ molecular ions of phosphatidylglycerol (PG) at *m/z* 743.5 with a characteristic product ion indicated by a grey circle at *m/z* 227, corresponding to glycerophosphate anion (**a**); and molecular ions of phosphatidylinositol (PI) at *m/z* 831.5 with a characteristic product ion indicated by a grey circle at *m/z* 241, corresponding to an inositol-1,2-cyclic phosphate anion, $[C_6H_{10}O_5PO_3]^-$ (**b**) [48].

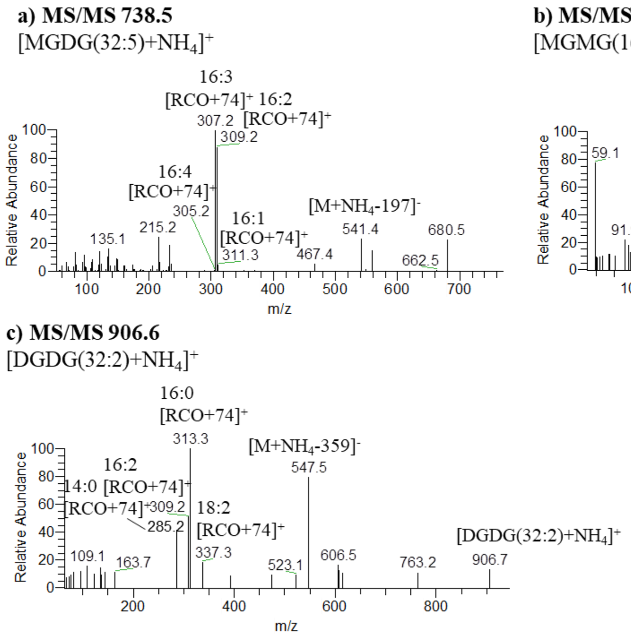


Figure S2.5. Example of HILIC-ESI–MS/MS spectra of the [M + NH$_4$]$^+$ molecular ions of monogalactosyldiacylglycerol (MGDG) at *m/z* 738.5, with characteristic combined neutral loss of NH_3 (-17 Da) and hexose (-180 Da) at *m/z* 197 (**a**), monogalactosylmonoacylglycerol (MGMG) at *m/z* 506.3 (**b**) and digalactosyldiacylglycerol (DGDG) at *m/z* 906.6 with characteristic combined neutral loss corresponding to the combined loss of NH_3 (-17 Da) and carbohydrate moiety (loss of a hexose 180 Da plus hexose residue 162 Da) at *m/z* 359 (**c**). In all the three spectra it is possible to observe the presence of the product ions corresponding to the fatty acyl side chain as an acylium ion plus 74 [RCO+74]$^+$ [22,48].

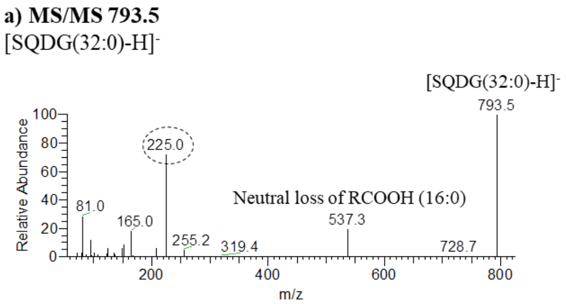


Figure S2.6. Example of a HILIC-ESI–MS/MS spectrum of the [M − H]$^-$ molecular ions of sulfoquinovosyldiacylglycerol (SQDG) at *m/z* 793.5 (**a**), with a characteristic product ion at *m/z*

225.0, corresponding to the anion of the sulfoquinovosyl polar head group and a neutral loss corresponding to the loss of fatty acyl chain as carboxylic acid (RCOOH) at *m/z* 256.2 [22,48].

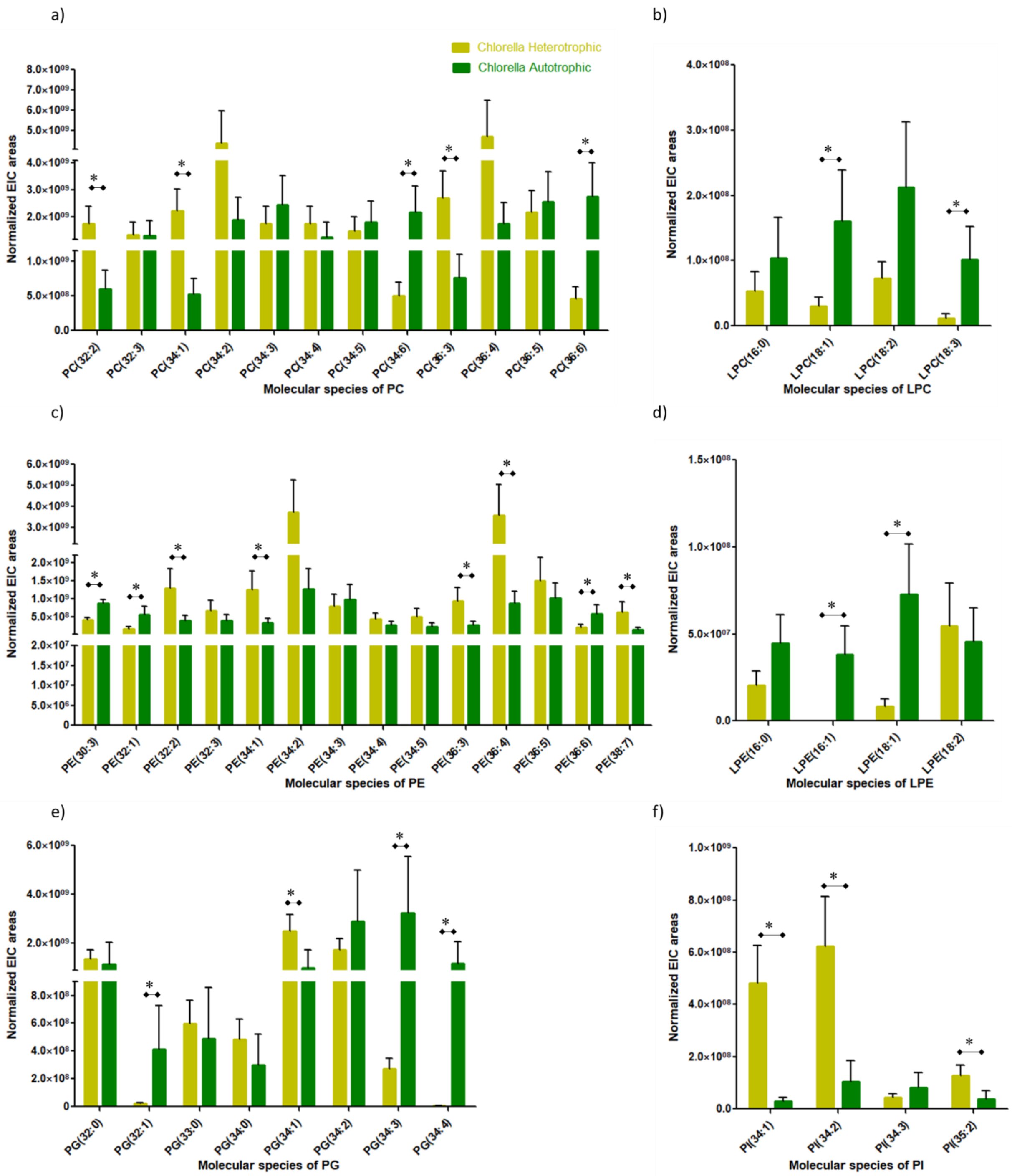


Figure S2.7. Most abundant phospholipids species of *Chlorella vulgaris* cultivated under heterotrophic conditions (yellow colour) and autotrophic conditions (green colour) determined by HILIC-ESI-MS: (**a**) phosphatidylcholine (PC); (**b**) lysophosphatidylcholine (LPC); (**c**) phosphatidylethanolamine (PE); (**d**) lysophosphatidylethanolamine (LPE); (**e**) phosphatidylglycerol (PG); and (**f**) phosphatidylinositol (PI). The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments. Statistic significant differences (*q*-value<0.05) are annotated with “*”.

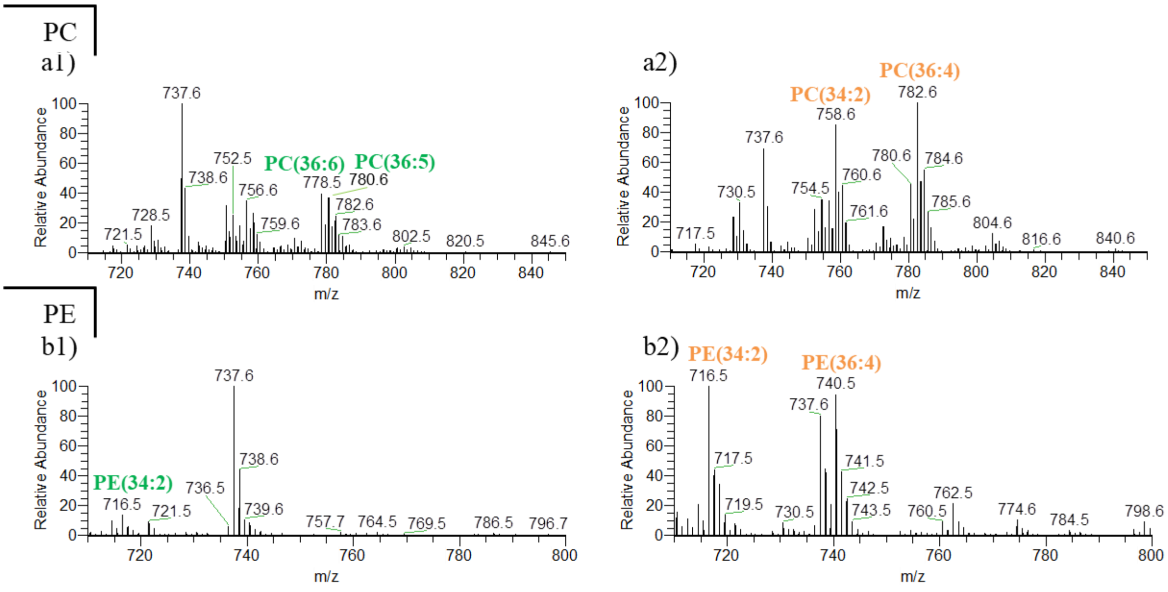


Figure S2.8. Comparison HILIC-ESI–MS spectra acquired in positive ion mode of *Chlorella vulgaris* of the phosphatidylcholine (PC) (**a1**, **a2**) and phosphatidylethanolamine (PE) (**b1**, **b2**) classes identified in the total lipid extracts of *Chlorella vulgaris* grown under autotrophic (**a1**, **b1**) and heterotrophic (**a2**, **b2**) conditions.

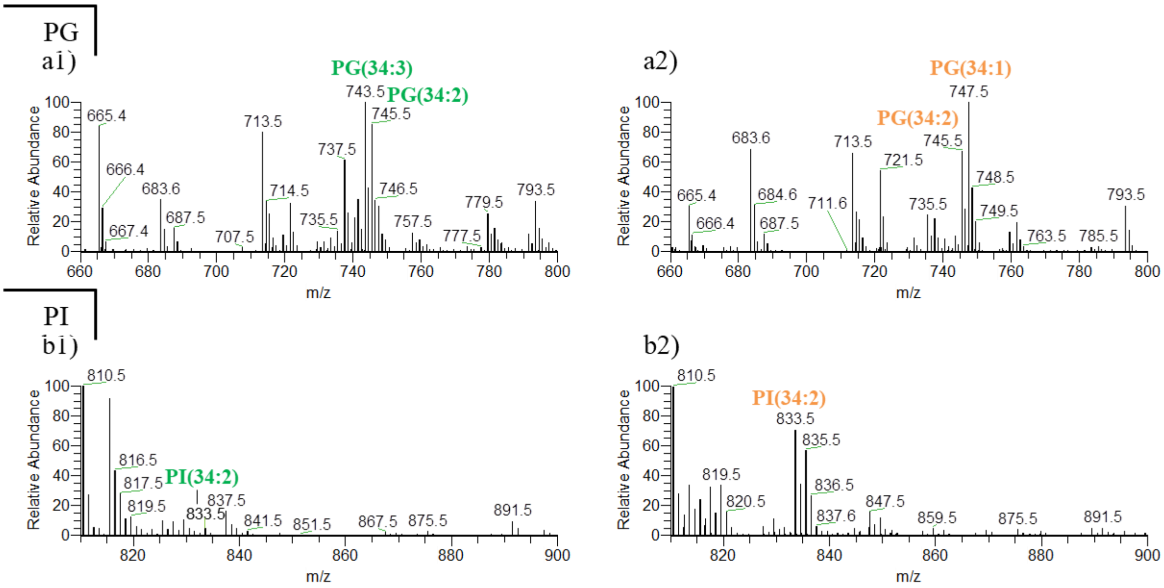


Figure S2.9. Comparison HILIC-ESI–MS spectra acquired in negative ion mode of *Chlorella vulgaris* of the phosphatidylglycerol (PG) (**a1**, **a2**), phosphatidylinositol (PI) and inositolphosphoceramide (PI-Cer) (**b1**, **b2**) classes identified in the total lipid extracts of *Chlorella vulgaris* grown under autotrophic (**a1**, **b1**) and heterotrophic (**a2**, **b2**) conditions.

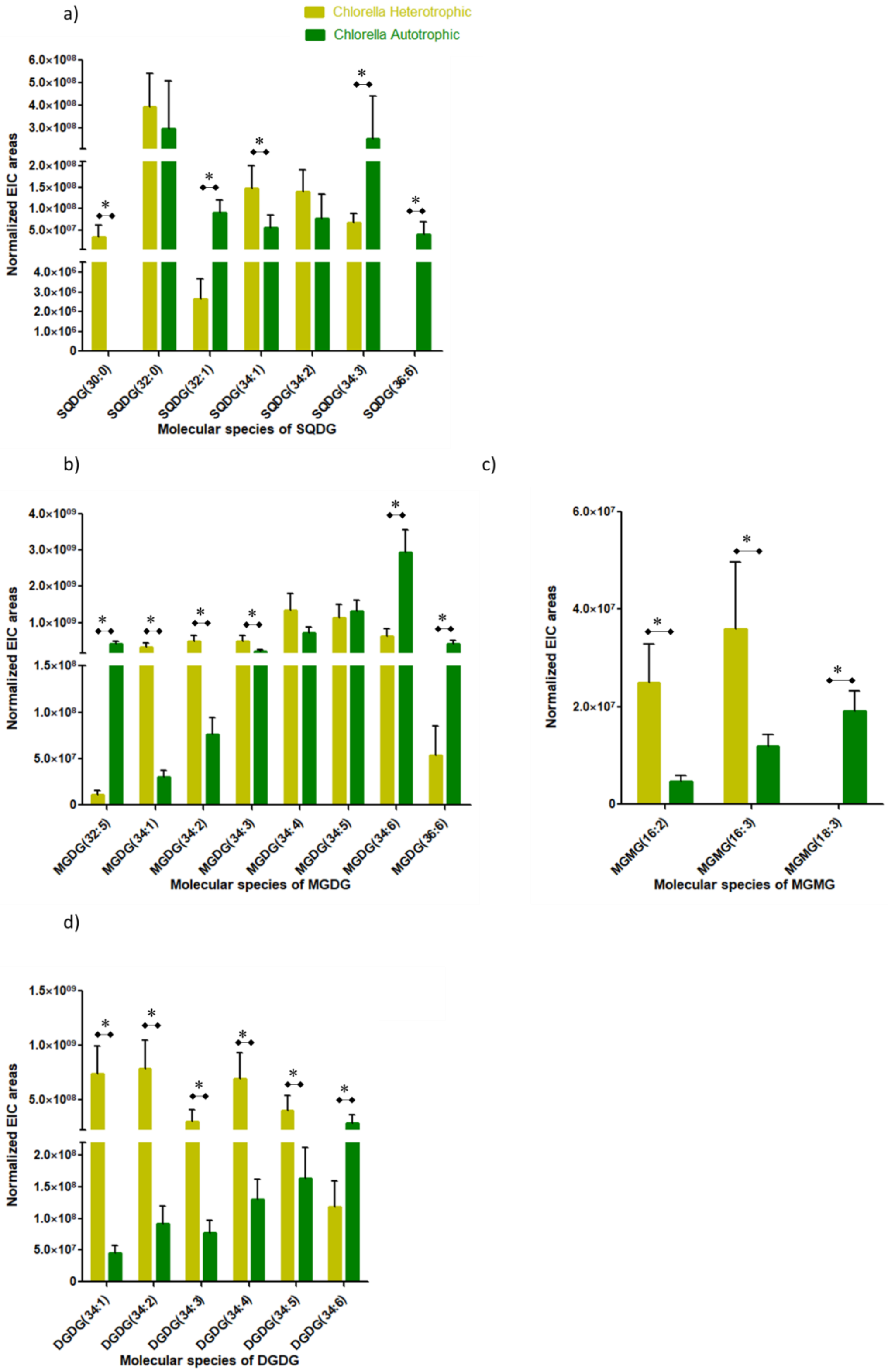


Figure S2.10. Most abundant glycolipid species of *Chlorella vulgaris* cultivated under heterotrophic conditions (yellow colour) and autotrophic conditions (green colour) determined by HILIC-ESI-MS:(**a**) sulfoquinovosyldiacylglycerol (SQDG); (**b**) monogalactosyldiacylglycerol (MGDG); (**c**) monogalactosylmonoacylglycerol (MGMG); (**d**) digalactosyldiacylglycerol (DGDG) lipid species. The numbers in parentheses (C:N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments. Statistically significant (*q*-value<0.05) are annotated with “*”.

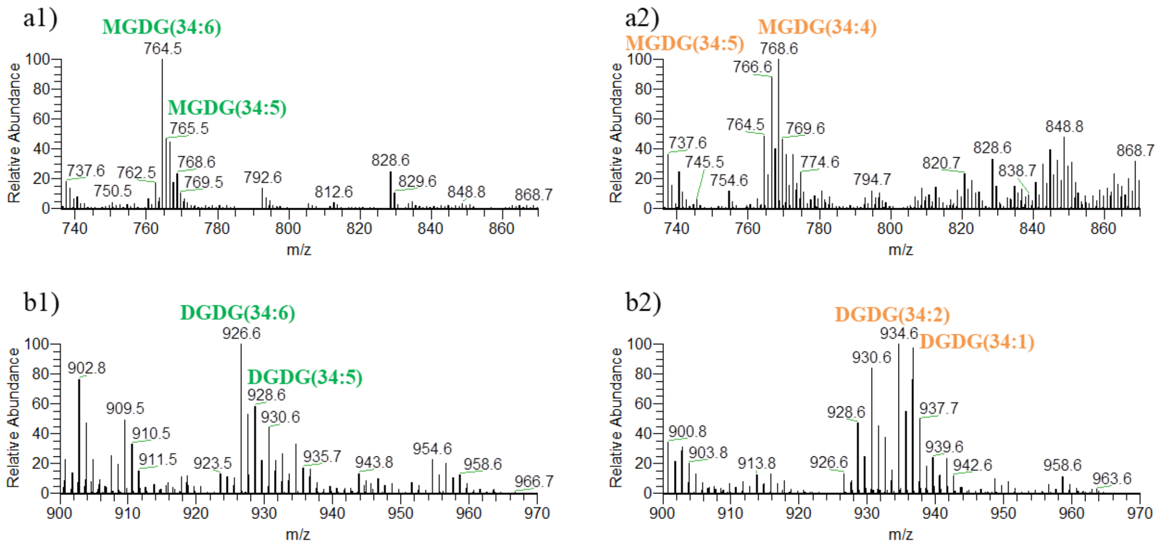


Figure S2.11. Comparison HILIC-ESI–MS spectra acquired in negative ion mode of *Chlorella vulgaris* of the monogalactosyldiacylglycerol (MGDG) (**a1**, **a2**) and digalactosyldiacylglycerol (DGDG) (**b1**, **b2**) classes grown under autotrophic (**a1**, **b1**) and heterotrophic (**a2**, **b2**) conditions.

Table S2.1. Exact mass measurements of the molecular ions identified by HILIC−ESI−MS in the total lipid extracts of *Chlorella vulgaris* grown under autotrophic and heterotrophic conditions. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains.

Lipid species (C:N)	**Formula**	**Calculated *m/z***	**Observed *m/z* of molecular ions in MS of the C-Auto**	**Error (ppm)**	**Observed *m/z* of molecular ions in MS of the C-Hetero**	**Error (ppm)**
			PC identified as $[M+H]^+$			
PC(30:3)	$C_{38}H_{71}NO_8P$	700.4917	700.4902	**-2.1870**	700.4902	**-2.1870**
PC(32:6)	$C_{40}H_{69}NO_8P$	722.4761	722.4764	**0.4402**	722.4774	**1.8243**
PC(32:5)	$C_{40}H_{71}NO_8P$	724.4917	724.4923	**0.7840**	724.4926	**1.1981**
PC(32:4)	$C_{40}H_{73}NO_8P$	726.5074	726.5077	**0.4377**	726.5079	**0.7130**
PC(32:3)	$C_{40}H_{75}NO_8P$	728.5230	728.5234	**0.5051**	728.5238	**1.0542**
PC(32:2)	$C_{40}H_{77}NO_8P$	730.5387	730.5385	**-0.2491**	730.5391	**0.5722**
PC(32:1)	$C_{40}H_{79}NO_8P$	732.5543	732.5528	**-2.0899**	732.5511	**-4.4106**
PC(32:0)	$C_{40}H_{81}NO_8P$	734.5700	734.5668	**-4.3318**	734.5689	**-1.4730**
PC(33:2)	$C_{41}H_{79}NO_8P$	744.5543	744.5542	**-0.1759**	744.5549	**0.7642**

PC(33:3)	$C_{41}H_{77}NO_8P$	742.5387	742.5389	**0.2936**	742.5397	**1.3710**
PC(34:8)	$C_{42}H_{69}NO_8P$	746.4761	746.4740	**-2.7891**	746.4745	**-2.1193**
PC(34:7)	$C_{42}H_{71}NO_8P$	748.4917	748.4901	**-2.1804**	748.4898	**-2.5812**
PC(34:6)	$C_{42}H_{73}NO_8P$	750.5074	750.5075	**0.1572**	750.5076	**0.2905**
PC(34:5(OH))	$C_{42}H_{75}NO_9P$	768.5179	768.5179	**-0.0612**	_	_
PC(34:5)	$C_{42}H_{75}NO_8P$	752.5230	752.5229	**-0.1754**	752.5232	**0.2232**
PC(34:4)	$C_{42}H_{77}NO_8P$	754.5387	754.5385	**-0.2412**	754.5390	**0.4214**
PC(34:3)	$C_{42}H_{79}NO_8P$	756.5543	756.5545	**0.2234**	756.5548	**0.6199**
PC(34:2)	$C_{42}H_{81}NO_8P$	758.5700	758.5699	**-0.1081**	758.5703	**0.4192**
PC(34:1)	$C_{42}H_{83}NO_8P$	760.5856	760.5833	**-3.0661**	760.5850	**-0.8309**
PC(35:1)	$C_{43}H_{85}NO_8P$	774.6013	774.5986	**-3.4624**	774.6008	**-0.6223**
PC(35:2)	$C_{43}H_{83}NO_8P$	772.5856	772.5849	**-0.9475**	772.5861	**0.6058**
PC(35:3)	$C_{43}H_{81}NO_8P$	770.5700	770.5700	**0.0234**	770.5701	**0.1531**
PC(35:4)	$C_{43}H_{79}NO_8P$	768.5543	768.5545	**0.2199**	768.5541	**-0.3006**
PC(36:9)	$C_{44}H_{71}NO_8P$	772.4917	772.4906	**-1.4654**	772.4919	**0.2175**
PC(36:8)	$C_{44}H_{73}NO_8P$	774.5074	774.5051	**-2.9464**	774.5060	**-1.7844**
PC(36:7)	$C_{44}H_{75}NO_8P$	776.5230	776.5200	**-3.9046**	776.5212	**-2.3592**
PC(36:6)	$C_{44}H_{77}NO_8P$	778.5387	778.5388	**0.1516**	778.5381	**-0.7476**
PC(36:5)	$C_{44}H_{79}NO_8P$	780.5543	780.5537	**-0.8084**	780.5543	**-0.0397**
PC(36:4(OH))	$C_{44}H_{81}NO_9P$	798.5649	798.5641	**-0.9980**	798.5649	**0.0038**
PC(36:4)	$C_{44}H_{81}NO_8P$	782.5700	782.5693	**-0.8715**	782.5701	**0.1508**
PC(36:3)	$C_{44}H_{83}NO_8P$	784.5856	784.5849	**-0.9330**	784.5851	**-0.6781**
PC(36:2)	$C_{44}H_{85}NO_8P$	786.6013	786.6008	**-0.6128**	786.6000	**-1.6298**
PC(36:1)	$C_{44}H_{87}NO_8P$	788.6169	788.6136	**-4.2251**	788.6149	**-2.5767**
PC(37:2)	$C_{45}H_{87}NO_8P$	800.6169	800.6155	**-1.7886**	800.6153	**-2.0384**
PC(37:3)	$C_{45}H_{85}NO_8P$	798.6013	798.6003	**-1.2296**	798.6010	**-0.3531**
PC(37:4)	$C_{45}H_{83}NO_8P$	796.5856	796.5854	**-0.2912**	796.5842	**-1.7977**
PC(37:5)	$C_{45}H_{81}NO_8P$	794.5700	794.5686	**-1.7393**	794.5670	**-3.7530**
PC(38:9)	$C_{46}H_{75}NO_8P$	800.5230	800.5215	**-1.9137**	800.5212	**-2.2885**
PC(38:8)	$C_{46}H_{77}NO_8P$	802.5387	802.5363	**-2.9681**	802.5373	**-1.7220**
PC(38:7)	$C_{46}H_{79}NO_8P$	804.5543	804.5522	**-2.6487**	804.5525	**-2.2758**
PC(38:6)	$C_{46}H_{81}NO_8P$	806.5700	806.5680	**-2.4573**	806.5671	**-3.5732**
PC(38:5)	$C_{46}H_{83}NO_8P$	808.5856	808.5861	**0.5788**	808.5825	**-3.8734**
PC(38:4)	$C_{46}H_{85}NO_8P$	810.6013	810.6025	**1.5026**	810.6027	**1.7493**
PC(38:3)	$C_{46}H_{87}NO_8P$	812.6169	812.6199	**3.6524**	812.6206	**4.5138**
PC(38:2)	$C_{46}H_{89}NO_8P$	814.6326	814.6341	**1.8634**	814.6342	**1.9862**
LPC identified as [M+H]$^+$						
LPC(16:3)	$C_{24}H_{45}NO_7P$	490.2934	490.2939	**1.0871**	_	_
LPC(16:2)	$C_{24}H_{47}NO_7P$	492.3090	492.3084	**-1.2533**	492.3082	**-1.6595**
LPC(16:1)	$C_{24}H_{49}NO_7P$	494.3247	494.3246	**-0.1355**	_	_
LPC(16:0)	$C_{24}H_{51}NO_7P$	496.3403	496.3405	**0.3707**	496.3407	**0.7737**
LPC(18:3)	$C_{26}H_{49}NO_7P$	518.3247	518.3249	**0.4495**	518.3244	**-0.5151**
LPC(18:2)	$C_{26}H_{51}NO_7P$	520.3403	520.3406	**0.5458**	520.3406	**0.5458**
LPC(18:1)	$C_{26}H_{53}NO_7P$	522.3560	522.3566	**1.2118**	522.3571	**2.1690**
LPC(18:0)	$C_{26}H_{55}NO_7P$	524.3716	524.3723	**1.3025**	_	_
LPC(20:5)	$C_{28}H_{49}NO_7P$	542.3247	542.3226	**-3.8114**	_	_

LPC(20:4)	$C_{28}H_{51}NO_7P$	544.3403	544.3393	**-1.8665**	_	_
PE identified as [M+H]$^+$						
PE(30:3)	$C_{35}H_{65}NO_8P$	658.4448	658.4427	**-3.1620**	658.4428	**-3.0101**
PE(30:2)	$C_{35}H_{67}NO_8P$	660.4604	660.4626	**3.2826**	660.4578	**-3.9851**
PE(30:1)	$C_{35}H_{69}NO_8P$	662.4761	662.4767	**0.9329**	_	_
PE(30:0)	$C_{35}H_{71}NO_8P$	664.4917	664.4921	**0.5538**	_	_
PE(32:6)	$C_{37}H_{63}NO_8P$	680.4291	_	_	680.4261	**-4.4560**
PE(32:5)	$C_{37}H_{65}NO_8P$	682.4448	_	_	682.4459	**1.6382**
PE(32:4)	$C_{37}H_{67}NO_8P$	684.4604	684.4594	**-1.5078**	684.4609	**0.6838**
PE(32:3)	$C_{37}H_{69}NO_8P$	686.4761	686.4769	**1.1916**	686.4765	**0.6089**
PE(32:2)	$C_{37}H_{71}O_8NP$	688.4917	688.4927	**1.4060**	688.4927	**1.4060**
PE(32:1)	$C_{37}H_{73}NO_8P$	690.5074	690.5079	**0.7502**	690.5044	**-4.3186**
PE(33:3)	$C_{38}H_{71}NO_8P$	700.4917	700.4930	**1.8102**	700.4933	**2.2384**
PE(34:6)	$C_{39}H_{67}NO_8P$	708.4604	708.4598	**-0.8921**	708.4592	**-1.7390**
PE(34:5)	$C_{39}H_{69}NO_8P$	710.4761	710.4758	**-0.3969**	710.4753	**-1.1007**
PE(34:4)	$C_{39}H_{71}NO_8P$	712.4917	712.4911	**-0.8870**	712.4923	**0.7972**
PE(34:3)	$C_{39}H_{73}O_8NP$	714.5074	714.5077	**0.4451**	714.5080	**0.8649**
PE(34:2)	$C_{39}H_{75}NO_8P$	716.5230	716.5233	**0.3740**	716.5237	**0.9323**
PE(34:1)	$C_{39}H_{77}NO_8P$	718.5387	718.5372	**-2.0625**	718.5379	**-1.0883**
PE(35:2)	$C_{40}H_{77}NO_8P$	730.5387	730.5387	**0.0246**	730.5397	**1.3935**
PE(35:3)	$C_{40}H_{75}NO_8P$	728.5230	728.5244	**1.8778**	728.5248	**2.4268**
PE(35:4)	$C_{40}H_{73}NO_8P$	726.5074	726.5078	**0.5754**	726.5082	**1.1259**
PE(36:9)	$C_{41}H_{65}NO_8P$	730.4448	730.4429	**-2.5765**	730.4419	**-3.9455**
PE(36:8)	$C_{41}H_{67}NO_8P$	732.4604	732.4573	**-4.2760**	732.4577	**-3.7299**
PE(36:7)	$C_{41}H_{69}NO_8P$	734.4761	734.4779	**2.4752**	734.4739	**-2.9708**
PE(36:6)	$C_{41}H_{71}NO_8P$	736.4917	736.4918	**0.0923**	736.4911	**-0.8581**
PE(36:5)	$C_{41}H_{73}NO_8P$	738.5074	738.5079	**0.7014**	738.5069	**-0.6527**
PE(36:4(OH))	$C_{41}H_{75}NO_9P$	756.5179	756.5182	**0.3344**	756.5186	**0.8632**
PE(36:4)	$C_{41}H_{75}NO_8P$	740.5230	740.5230	**-0.0432**	740.5231	**0.0918**
PE(36:3)	$C_{41}H_{77}NO_8P$	742.5387	742.5378	**-1.1878**	742.5369	**-2.3999**
PE(36:2)	$C_{41}H_{79}O_8NP$	744.5543	744.5552	**1.1671**	744.5559	**2.1073**
PE(37:5)	$C_{42}H_{75}NO_8P$	752.5230	752.5226	**-0.5741**	752.5228	**-0.3083**
PE(38:9)	$C_{43}H_{69}NO_8P$	758.4761	758.4733	**-3.6679**	758.4731	**-3.9316**
PE(38:8)	$C_{43}H_{71}NO_8P$	760.4917	760.4886	**-4.1184**	760.4887	**-3.9869**
PE(38:7)	$C_{43}H_{73}NO_8P$	762.5074	762.5045	**-3.7796**	762.5053	**-2.7305**
LPE identified as [M+H]$^+$						
LPE(16:1)	$C_{21}H_{43}NO_7P$	452.2777	452.2775	**-0.4798**	_	_
LPE(16:0)	$C_{21}H_{45}NO_7P$	454.2934	454.2930	**-0.8078**	454.2931	**-0.5877**
LPE(18:3)	$C_{23}H_{43}NO_7P$	476.2777	476.2778	**0.1743**	_	_
LPE(18:2)	$C_{23}H_{45}NO_7P$	478.2934	478.2933	**-0.1401**	478.2936	**0.4871**
LPE(18:1)	$C_{23}H_{47}NO_7P$	480.3090	480.3093	**0.5892**	480.3091	**0.1728**
LPE(20:5)	$C_{25}H_{43}NO_7P$	500.2777	500.2756	**-4.2316**	500.2757	**-4.0318**
LPE(20:4)	$C_{25}H_{45}NO_7P$	502.2934	502.2915	**-3.7170**	_	_
PG identified as [M-H]$^-$						
PG(32:1)	$C_{38}H_{72}O_{10}P$	719.4863	719.4884	**2.9187**	719.4868	**0.6949**
PG(32:0)	$C_{38}H_{74}O_{10}P$	721.5020	721.5039	**2.6334**	721.5043	**3.1878**

PG(33:0)	$C_{39}H_{76}O_{10}P$	735.5176	735.5199	**3.1094**	735.5199	**3.1094**
PG(34:4)	$C_{40}H_{70}O_{10}P$	741.4707	741.4724	**2.2927**	741.4700	**-0.9441**
PG(34:3)	$C_{40}H_{72}O_{10}P$	743.4863	743.4883	**2.6900**	743.4885	**2.9590**
PG(34:2)	$C_{40}H_{74}O_{10}P$	745.5020	745.5041	**2.8169**	745.5043	**3.0852**
PG(34:1)	$C_{40}H_{76}O_{10}P$	747.5176	747.5186	**1.3378**	747.5198	**2.9431**
PG(34:0)	$C_{40}H_{78}O_{10}P$	749.5333	749.5338	**0.6671**	749.5316	**-2.2681**
PG(36:5)	$C_{42}H_{72}O_{10}P$	767.4863	767.4878	**1.9544**	767.4875	**1.5635**
PG(36:4)	$C_{42}H_{74}O_{10}P$	769.5020	769.5023	**0.3899**	769.5036	**2.0793**
PG(36:3)	$C_{42}H_{76}O_{10}P$	771.5176	771.5196	**2.5923**	771.5189	**1.6850**
PG(36:2)	$C_{42}H_{78}O_{10}P$	773.5333	773.5351	**2.3270**	773.5344	**1.4220**
PG(36:1)	$C_{42}H_{80}O_{10}P$	775.5489	775.5498	**1.1605**	775.5507	**2.3209**
PG(36:0)	$C_{42}H_{82}O_{10}P$	777.5646	777.5654	**1.0289**	777.5644	**-0.2572**
PG(38:5)	$C_{44}H_{76}O_{10}P$	795.5176	795.5183	**0.8799**	795.5210	**4.2739**
PI identified as [M-H]$^-$						
PI(32:2)	$C_{41}H_{74}O_{13}P$	805.4867	_	_	805.4847	**-2.4830**
PI(34:3)	$C_{43}H_{76}O_{13}P$	831.5024	831.5034	**1.2026**	831.5025	**0.1203**
PI(34:2)	$C_{43}H_{78}O_{13}P$	833.5180	833.5139	**-4.9189**	833.5198	**2.1595**
PI(34:1)	$C_{43}H_{80}O_{13}P$	835.5337	835.5308	**-3.4708**	835.5344	**0.8378**
PI(35:2)	$C_{44}H_{80}O_{13}P$	847.5337	847.5337	**0.0496**	847.5353	**1.9374**
PI(36:5)	$C_{45}H_{76}O_{13}P$	855.5024	855.5028	**0.4676**	855.5035	**1.2858**
PI(36:4)	$C_{45}H_{78}O_{13}P$	857.5180	_	_	857.5197	**1.9825**
PI(36:3)	$C_{45}H_{80}O_{13}P$	859.5337	_	_	859.5345	**0.9307**
PI(36:2)	$C_{45}H_{82}O_{13}P$	861.5493	_	_	861.5492	**-0.1161**
PI-Cer identified as [M-H]$^-$						
PI-Cer(t34:0(OH))	$C_{40}H_{79}NO_{13}P$	812.5289	812.5295	**0.7298**	812.5271	**-2.2239**
Cer identified as [M+H]$^+$						
Cer(d35:0)	$C_{35}H_{72}NO_3$	554.5512	554.5512	**-0.0343**	554.5518	**1.0477**
MGMG identified as [M+NH$_4$]$^+$						
MGMG(16:3)	$C_{25}H_{46}NO_9$	504.3173	504.3176	**0.6762**	504.3181	**1.6676**
MGMG(16:2)	$C_{25}H_{48}NO_9$	506.3329	506.3329	**-0.0178**	506.3327	**-0.4128**
MGMG(16:0)	$C_{25}H_{52}NO_9$	510.3642	_	_	510.3635	**-1.3716**
MGMG(18:3)	$C_{27}H_{50}NO_9$	532.3486	532.3491	**1.0163**	_	_
MGMG(18:2)	$C_{27}H_{52}NO_9$	534.3642	534.3646	**0.7317**	534.3652	**1.8545**
MGDG identified as [M+NH$_4$]$^+$						
MGDG(32:6)	$C_{41}H_{70}NO_{10}$	736.4994	736.5008	**1.9009**	_	_
MGDG(32:5)	$C_{41}H_{72}NO_{10}$	738.5156	738.5165	**1.1862**	738.5176	**2.6756**
MGDG(32:4)	$C_{41}H_{74}NO_{10}$	740.5307	740.5328	**2.8358**	740.5340	**4.4563**
MGDG(32:3)	$C_{41}H_{76}NO_{10}$	742.5464	742.5474	**1.3467**	742.5496	**4.3095**
MGDG(32:2)	$C_{41}H_{78}NO_{10}$	744.5626	744.5615	**-1.4774**	744.5629	**0.4029**
MGDG(34:7)	$C_{43}H_{72}NO_{10}$	762.5156	_	_	762.5182	**3.3783**
MGDG(34:6)	$C_{43}H_{74}NO_{10}$	764.5313	764.5327	**1.8665**	764.5317	**0.5585**
MGDG(34:5)	$C_{43}H_{76}NO_{10}$	766.5469	766.5463	**-0.8140**	766.5507	**4.9260**
MGDG(34:4)	$C_{43}H_{78}NO_{10}$	768.5626	768.5626	**0.0000**	768.5637	**1.4312**
MGDG(34:3)	$C_{43}H_{80}NO_{10}$	770.5782	770.5770	**-1.5884**	770.5779	**-0.4205**
MGDG(34:2)	$C_{43}H_{82}NO_{10}$	772.5933	772.5936	**0.3883**	772.5946	**1.6826**
MGDG(34:1)	$C_{43}H_{84}NO_{10}$	774.6090	774.6097	**0.9037**	774.6099	**1.1619**

MGDG(34:0)	$C_{43}H_{86}NO_{10}$	776.6246	776.6242	**-0.5150**	776.6211	**-4.5067**
MGDG(35:1)	$C_{44}H_{86}NO_{10}$	788.6252	788.6240	**-1.4887**	788.6256	**0.5402**
MGDG(36:6)	$C_{45}H_{78}NO_{10}$	792.5625	792.5632	**0.8832**	792.5652	**3.4067**
MGDG(36:5)	$C_{45}H_{80}NO_{10}$	794.5782	794.5770	**-1.5102**	794.5802	**2.5171**
MGDG(36:4)	$C_{45}H_{82}NO_{10}$	796.5933	796.5933	**0.0000**	796.5946	**1.6319**
MGDG(36:3)	$C_{45}H_{84}NO_{10}$	798.6095	798.6070	**-3.1555**	798.6082	**-1.6529**
MGDG(36:2)	$C_{45}H_{86}NO_{10}$	800.6252	800.6242	**-1.2165**	800.6229	**-2.8403**
DGMG identified as [M+NH$_4$]$^+$						
DGMG(16:0)	$C_{31}H_{62}O_{14}N$	672.4170	672.4170	**0.0000**	672.4175	**0.7436**
DGDG identified as [M+NH$_4$]$^+$						
DGDG(28:0)	$C_{43}H_{84}O_{15}N$	854.5841	854.5840	**-0.1170**	854.5815	**-3.0424**
DGDG(32:5)	$C_{47}H_{82}O_{15}N$	900.5684	900.5699	**1.6656**	_	_
DGDG(32:4)	$C_{47}H_{84}O_{15}N$	902.5841	902.5844	**0.3324**	_	_
DGDG(32:3)	$C_{47}H_{86}O_{15}N$	904.5997	904.6015	**1.9898**	904.6015	**1.9898**
DGDG(32:2)	$C_{47}H_{88}O_{15}N$	906.6154	906.6153	**-0.1103**	906.6160	**0.6618**
DGDG(32:1)	$C_{47}H_{90}O_{15}N$	908.6310	908.6298	**-1.3207**	908.6285	**-2.7514**
DGDG(32:0)	$C_{47}H_{92}O_{15}N$	910.6467	910.6469	**0.2196**	910.6480	**1.4276**
DGDG(34:6)	$C_{49}H_{84}O_{15}N$	926.5841	926.5852	**1.1872**	926.5859	**1.9426**
DGDG(34:5)	$C_{49}H_{86}O_{15}N$	928.5997	928.5989	**-0.8615**	928.6010	**1.4000**
DGDG(34:4)	$C_{49}H_{88}O_{15}N$	930.6154	930.6162	**0.8596**	930.6162	**0.8596**
DGDG(34:3)	$C_{49}H_{90}O_{15}N$	932.6310	932.6305	**-0.5361**	932.6299	**-1.1795**
DGDG(34:2)	$C_{49}H_{92}O_{15}N$	934.6467	934.6471	**0.4280**	934.6477	**1.0699**
DGDG(34:1)	$C_{49}H_{94}O_{15}N$	936.6623	936.6609	**-1.4947**	936.6624	**0.1068**
DGDG(35:1)	$C_{50}H_{96}O_{15}N$	950.6780	950.6759	**-2.2079**	950.6782	**0.2114**
DGDG(35:2)	$C_{50}H_{94}O_{15}N$	948.6623	948.6625	**0.1592**	948.6638	**1.5295**
DGDG(35:3)	$C_{50}H_{92}O_{15}N$	946.6467	946.6484	**1.7969**	946.6485	**1.9025**
DGDG(36:7)	$C_{51}H_{86}O_{15}N$	952.5997	952.6010	**1.3647**	952.6024	**2.8343**
DGDG(36:6)	$C_{51}H_{88}O_{15}N$	954.6154	954.6173	**1.9903**	954.6198	**4.6092**
DGDG(36:5)	$C_{51}H_{90}O_{15}N$	956.6310	956.6315	**0.5227**	956.6330	**2.0907**
DGDG(36:4)	$C_{51}H_{92}O_{15}N$	958.6467	958.6458	**-0.9388**	958.6476	**0.9388**
DGDG(36:3)	$C_{51}H_{94}O_{15}N$	960.6623	960.6593	**-3.1228**	960.6601	**-2.2901**
DGDG(36:2)	$C_{51}H_{96}O_{15}N$	962.6780	_	_	962.6779	**-0.1039**
SQDG identified as [M-H]$^-$						
SQDG(30:0)	$C_{39}H_{73}O_{12}S$	765.4823	_	_	765.4824	**0.1607**
SQDG(32:3)	$C_{41}H_{71}O_{12}S$	787.4666	787.4692	**3.2674**	787.4700	**4.2834**
SQDG(32:2)	$C_{41}H_{73}O_{12}S$	789.4823	789.4829	**0.7891**	789.4840	**2.1824**
SQDG(32:1)	$C_{41}H_{75}O_{12}S$	791.4979	791.4975	**-0.5395**	791.4995	**1.9874**
SQDG(32:0)	$C_{41}H_{77}O_{12}S$	793.5136	793.5151	**1.9206**	793.5157	**2.6767**
SQDG(34:5)	$C_{43}H_{71}O_{12}S$	811.4666	811.4698	**3.9102**	811.4683	**2.0617**
SQDG(34:4)	$C_{43}H_{73}O_{12}S$	813.4823	813.4844	**2.6098**	813.4843	**2.4868**
SQDG(34:3)	$C_{43}H_{75}O_{12}S$	815.4979	815.5004	**3.0325**	815.5003	**2.9099**
SQDG(34:2)	$C_{43}H_{77}O_{12}S$	817.5136	817.5149	**1.6195**	817.5162	**3.2097**
SQDG(34:1)	$C_{43}H_{79}O_{12}S$	819.5292	819.5304	**1.4313**	819.5315	**2.7735**
SQDG(34:0)	$C_{43}H_{81}O_{12}S$	821.5449	821.5445	**-0.4589**	821.5420	**-3.5019**
SQDG(36:6)	$C_{45}H_{73}O_{12}S$	837.4823	837.4851	**3.3708**	_	_
SQDG(36:5)	$C_{45}H_{75}O_{12}S$	839.4979	839.5013	**4.0179**	839.5000	**2.4693**

SQDG(36:4)	$C_{45}H_{77}O_{12}S$	841.5136	841.5162	**3.1182**	841.5173	**4.4254**
SQDG(36:3)	$C_{45}H_{79}O_{12}S$	843.5292	843.5326	**3.9987**	843.5323	**3.6430**

C-Auto, *Chlorella vulgaris* cultivated under autotrophic conditions, C-Hetero, *Chlorella vulgaris* cultivated under heterotrophic conditions, PC, phosphatidylcholine, LPC, lysophosphatidylcholine, PE, phosphatidylethanolamine, LPE, lysophosphatidylethanolamine, PG, phosphatidylglycerol, PI, phosphatidylinositol, DGDG, digalactosyldiacylglycerol, DGMG, digalactosylmonoacylglycerol, MGDG, monogalactosyldiacylglycerol, MGMG, monogalactosylmonoacylglycerol, SQDG, sulfoquinovosyldiacylglycerol, Cer, Ceramide, PI_Cer, inositolphosphoceramide.

Table S2.2. Average peak area of the all lipid species identified by HILIC−ESI−MS in the total lipid extracts of *Chlorella vulgaris* grown under autotrophic and heterotrophic conditions. C represents the total number of carbon atoms and N the total number of double bonds on fatty acyl chains.

Lipid species (C:N)	**Mean area of peaks (C-Hetero)**	**Mean area of peaks (C-Auto)**
Cer(d35:0)	4245154.0	4556869.8
DGDG(28:0)	1787640.0	863963.7
DGDG(32:0)	2794689.3	937451.1
DGDG(32:1)	502684.0	598920.4
DGDG(32:2)	2174564.0	1651263.7
DGDG(32:3)	718831.5	2557627.4
DGDG(32:4)	NA	2399179.7
DGDG(32:5)	NA	2638928.2
DGDG(34:1)	58162167.7	5399953.5
DGDG(34:2)	61883423.2	11032115.9
DGDG(34:3)	23535638.5	9134826.1
DGDG(34:4)	54407056.6	15468145.8
DGDG(34:5)	31199930.2	19582010.7
DGDG(34:6)	9310096.4	33408096.3
DGDG(35:1)	4425376.8	277839.6
DGDG(35:2)	5461660.5	2125801.7
DGDG(35:3)	1751647.4	3368671.6
DGDG(36:2)	451865.9	NA
DGDG(36:3)	2175243.1	751688.7
DGDG(36:4)	7144924.8	3934806.7
DGDG(36:5)	3903220.4	6523361.6
DGDG(36:6)	2303554.0	7406925.5
DGDG(36:7)	299180.0	1602036.5
DGMG(16:0)	176371.0	126385.9
LPC(16:0)	2503859.6	3191331.4
LPC(16:1)	NA	612208.7
LPC(16:2)	495591.3	639260.9
LPC(16:3)	NA	843336.1
LPC(18:0)	NA	395621.0
LPC(18:1)	1420652.0	4977873.1

LPC(18:2)	3357830.3	6584218.5
LPC(18:3)	567024.8	3142784.8
LPC(20:4)	NA	805420.4
LPC(20:5)	NA	586810.3
LPE(16:0)	935430.5	1384855.8
LPE(16:1)	NA	1168627.5
LPE(18:1)	382626.2	2244127.8
LPE(18:2)	2529674.6	1398268.8
LPE(18:3)	NA	506469.5
LPE(20:4)	NA	521429.4
LPE(20:5)	426207.7	204694.5
MGDG(32:2)	3150523.5	3466323.8
MGDG(32:3)	6219514.1	12981345.0
MGDG(32:4)	8674684.4	28998076.8
MGDG(32:5)	860480.8	47971022.0
MGDG(32:6)	NA	6749543.0
MGDG(34:0)	3385808.5	835365.8
MGDG(34:1)	25379935.3	3633832.2
MGDG(34:2)	37644649.2	9087038.7
MGDG(34:3)	37600019.5	23686791.6
MGDG(34:4)	106095563.4	86029024.3
MGDG(34:5)	89844293.6	157385600.5
MGDG(34:6)	48808748.8	350971832.3
MGDG(34:7)	2482380.8	NA
MGDG(35:1)	2465927.5	684239.8
MGDG(36:2)	1073585.2	253946.1
MGDG(36:3)	4279783.9	2183493.7
MGDG(36:4)	8117626.7	7149185.4
MGDG(36:5)	5522915.2	18967027.0
MGDG(36:6)	4140458.7	49971251.8
MGMG(16:0)	483407.7	NA
MGMG(16:2)	1954147.8	546052.8
MGMG(16:3)	2757707.5	1426072.0
MGMG(18:2)	165316.3	368667.5
MGMG(18:3)	NA	2274839.6
PC(30:3)	14858116.1	20460723.8
PC(32:0)	1783490.9	984744.7
PC(32:1)	12978852.8	9450392.2
PC(32:2)	80761827.2	18720823.3
PC(32:3)	61177033.4	39919355.2
PC(32:4)	5664922.6	10368310.0
PC(32:5)	4637142.8	10738909.4
PC(32:6)	1093112.1	6183929.7
PC(33:2)	16105700.0	10934342.9
PC(33:3)	9778143.7	16516126.4
PC(34:1)	102600525.6	16199428.2

PC(34:2)	200813584.1	58199414.1
PC(34:3)	79949444.8	75446755.4
PC(34:4)	80368688.6	38474549.5
PC(34:5(OH))	NA	973468.4
PC(34:5)	67732749.6	55610354.9
PC(34:6)	23266477.5	66992771.9
PC(34:7)	468476.8	1535139.5
PC(34:8)	314781.8	1223435.6
PC(35:1)	21162476.6	4860271.5
PC(35:2)	37812206.5	16603778.5
PC(35:3)	14410467.4	21281718.6
PC(35:4)	4381925.6	11993252.1
PC(36:1)	6207394.6	2468291.4
PC(36:2)	36074885.2	11508738.1
PC(36:3)	123852205.7	23658012.3
PC(36:4(OH))	1408712.9	2434350.0
PC(36:4)	217042328.0	54077129.3
PC(36:5)	99450711.6	78693533.6
PC(36:6)	21131928.4	85234738.9
PC(36:7)	10097134.7	6011385.5
PC(36:8)	6715049.9	6680936.8
PC(36:9)	1696412.3	7730355.5
PC(37:2)	3433586.5	2072980.0
PC(37:3)	9147161.9	4123619.4
PC(37:4)	5370663.0	5455981.0
PC(37:5)	5136585.0	3491466.0
PC(38:2)	1062203.6	593638.5
PC(38:3)	2049200.6	705902.2
PC(38:4)	1386160.3	849221.4
PC(38:5)	4721115.9	1893622.7
PC(38:6)	15207795.5	3618720.7
PC(38:7)	25775633.9	7748997.9
PC(38:8)	8893999.9	9575666.3
PC(38:9)	1539417.1	9434959.2
PE(30:0)	NA	4386941.4
PE(30:1)	NA	2173125.9
PE(30:2)	329682.8	471296.4
PE(30:3)	18881059.2	27515472.4
PE(32:1)	7784949.6	17203412.7
PE(32:2)	59330601.1	12243590.8
PE(32:3)	30779727.1	12222623.9
PE(32:4)	565860.8	1148861.0
PE(32:5)	257837.3	NA
PE(32:6)	2631659.0	NA
PE(33:3)	4736891.8	5976712.1
PE(34:1)	57366431.8	10596610.2

PE(34:2)	171164021.9	39474162.0
PE(34:3)	36211320.4	30053176.6
PE(34:4)	19977713.9	8526920.9
PE(34:5)	23636421.4	7412060.8
PE(34:6)	9487960.1	8347497.1
PE(35:2)	14470084.3	7238805.1
PE(35:3)	4452497.0	6244401.7
PE(35:4)	1865269.4	4196786.9
PE(36:2)	8736292.0	5376737.9
PE(36:3)	42837933.7	8415329.7
PE(36:4(OH))	1175341.4	952203.0
PE(36:4)	164303365.1	26866890.5
PE(36:5)	68804515.7	31639648.9
PE(36:6)	9554331.3	18440838.9
PE(36:7)	3103467.4	1196614.2
PE(36:8)	3829695.2	1116849.3
PE(36:9)	1331436.3	1285966.2
PE(37:5)	4204275.6	2464320.3
PE(38:7)	28913829.8	4717828.6
PE(38:8)	11287392.1	5216660.4
PE(38:9)	1398912.1	3026919.3
PG(32:0)	62605599.8	35499578.1
PG(32:1)	990838.3	12627597.3
PG(33:0)	27580980.7	15047208.8
PG(34:0)	22439537.3	9159942.4
PG(34:1)	114923354.5	30642049.3
PG(34:2)	79291100.2	89006172.1
PG(34:3)	12525546.3	99144574.0
PG(34:4)	271217.8	36390293.2
PG(36.5)	542513.0	444162.7
PG(36:0)	467968.9	864351.2
PG(36:1)	1732832.3	1933313.6
PG(36:2)	1204672.1	3465990.2
PG(36:3)	1028556.9	1368885.2
PG(36:4)	1137173.2	1319638.7
PG(38:5)	4044989.6	5833375.7
PI(32.2)	2280080.8	NA
PI(34:1)	22260106.6	883409.7
PI(34.2)	28785313.0	3178188.3
PI(34.3)	2008124.1	2470913.6
PI(35:2)	5942399.3	1185522.9
PI(36:2)	1363353.5	NA
PI(36:3)	1819412.9	NA
PI(36:4)	1239542.8	NA
PI(36.5)	231941.4	225357.6
PI_Cer(t18:0/16:0(2OH))	4708666.2	5410163.4

SQDG(30:0)	2757983.2	NA
SQDG(32:0)	31311547.9	35911224.0
SQDG(32:1)	212799.8	10921436.8
SQDG(32:2)	453648.3	1633457.5
SQDG(32:3)	96867.3	1896148.4
SQDG(34:0)	1702900.2	3015199.0
SQDG(34:1)	11730542.0	6779463.0
SQDG(34:2)	11177984.3	9299891.5
SQDG(34:3)	5322231.5	30452046.8
SQDG(34:4)	233098.1	1234394.2
SQDG(34:5)	73304.9	1017122.1
SQDG(36:3)	516251.2	154743.1
SQDG(36:4)	581677.4	668482.8
SQDG(36:5)	239860.2	1588459.2
SQDG(36:6)	NA	4818537.3

C-Auto, *Chlorella vulgaris* cultivated under autotrophic conditions, C-Hetero, *Chlorella vulgaris* cultivated under heterotrophic conditions, PC, phosphatidylcholine, LPC, lysophosphatidylcholine, PE, phosphatidylethanolamine, LPE, lysophosphatidylethanolamine, PG, phosphatidylglycerol, PI, phosphatidylinositol, DGDG, digalactosyldiacylglycerol, DGMG, digalactosylmonoacylglycerol, MGDG, monogalactosyldiacylglycerol, MGMG, monogalactosylmonoacylglycerol, SQDG, sulfoquinovosyldiacylglycerol, Cer, ceramide, PI_Cer, inositolphosphoceramide, NA, not available.

Table S2.3. List of molecular ions and lipid species of phosphatidylcholine (PC), lysophosphatidylcholine (LPC), ceramide (Cer), phosphatidylethanolamine (PE) and lysophosphatidylethanolamine (LPE) identified by HILIC-ESI-MS in positive ion mode, as $[M+H]^+$ ions, in the total lipid extracts of *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions. When possible, the identification of the fatty acyl composition correspondent to each molecular ion was confirmed by the analysis of the LC-MS/MS spectra of each $[M+CH_3COO]^-$ ion for PC and LPC class and $[M-H]^-$ for PE and LPE class. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains, bold *m/z* values correspond to the most abundant molecular ions in each class detected.

$[M + H]^+$	**Observed *m/z* of molecular ions in MS of the C-Auto**	**Lipid species (C:N) of the C-Auto**	**Fatty acyl chains observed in MS/MS of the C-Auto***	**Observed *m/z* of molecular ions in MS of the C-Hetero**	**Lipid species (C:N) of the C-Hetero**	**Fatty acyl chains observed in MS/MS of the C-Hetero***
PC	700.4902	PC(30:3)	(14:0/16:3)	700.4902	PC(30:3)[a]	
	722.4764	PC(32:6)	(16:3/16:3)	722.4774	PC(32:6)	(16:3/16:3)
	724.4923	PC(32:5)	(16:2/16:3)	724.4926	PC(32:5)	(16:2/16:3)
	726.5077	PC(32:4)	(16:2/16:2) and (16:1/16:3)	726.5079	PC(32:4)	(16:2/16:2) and (16:1/16:3)
	728.5234	PC(32:3)	(16:0/16:3)	728.5238	PC(32:3)	(16:0/16:3)
	730.5385	PC(32:2)[a]		730.5391	PC(32:2)	(16:0/16:2)
	732.5528	PC(32:1)	(16:0/16:1), (14:0/18:1) and (15:0/17:1)	732.5511	PC(32:1)[a]	
	734.5668	PC(32:0)	(16:0/16:0)	734.5689	PC(32:0)	(16:0/16:0)
	744.5542	PC(33:2)[b]		744.5549	PC(33:2)	(17:0/16:2) and (15:0/18:2)
	742.5389	PC(33:3)	(17:0/16:3), (17:1/16:2) and (15:0/18:3)	742.5397	PC(33:3)	(17:0/16:3), (17:1/16:2) and (15:0/18:3)
	746.474	PC(34:8)[b]		746.4745	PC(34:8)[b]	
	748.4901	PC(34:7)[b]		748.4898	PC(34:7)[a]	
	750.5075	PC(34:6)	(16:3/18:3)	750.5076	PC(34:6)	(16:3/18:3)
	768.5179	PC(34:5(OH))	(18:2/16:3-OH)	–	–	
	752.5229	PC(34:5)	(16:2/18:3) and (18:2/16:3)	752.5232	PC(34:5)	(16:2/18:3) and (18:2/16:3)
	754.5385	PC(34:4)	(18:1/16:3); (16:2/18:2) and (16:1/18:3)	754.539	PC(34:4)	(18:1/16:3); (16:2/18:2) and (16:1/18:3)

756.5545	PC(34:3)	(18:1/16:2); (16:1/18:2) and (16:0/18:3)	756.5548	PC(34:3)	(18:1/16:2); (16:1/18:2) and (16:0/18:3)
758.5699	PC(34:2)[a]		**758.5703**	**PC(34:2)**	**(16:0/18:2)**
760.5833	PC(34:1)	(16:0/18:1) and (17:0/17:1)	760.585	PC(34:1)	(16:0/18:1) and (17:0/17:1)
774.5986	PC(35:1)[b]		774.6008	PC(35:1)[b]	
772.5849	PC(35:2)[b]		772.5861	PC(35:2)	(17:0/18:2)
770.57	PC(35:3)	(17:0/18:3)	770.5701	PC(35:3)	(17:0/18:3)
768.5545	PC(35:4)	(17:2/18:2) and (17:1/18:3)	768.5541	PC(35:4)	(17:2/18:2) and (17:1/18:3)
772.4906	PC(36:9)[b]		772.4919	PC(36:9)[b]	
774.5051	PC(36:8)[b]		774.506	PC(36:8)[b]	
776.52	PC(36:7)[b]	(16:3/20:4)	776.5212	PC(36:7)[b]	
778.5388	**PC(36:6)**	**(18:3/18:3)**	778.5381	PC(36:6)	(18:3/18:3)
780.5537	**PC(36:5)**	**(18:2/18:3)**	780.5543	PC(36:5)	(18:2/18.3)
798.5641	PC(36:4(OH))	(18:2/18:2-OH)	798.5649	PC(36:4(OH))	(18:2/18:2-OH)
782.5693	PC(36:4)	(18:1/18:3) and (18:2/18:2)	**782.5701**	**PC(36:4)**	**(18:1/18:3) and (18:2/18:2)**
784.5849	PC(36:3)	(18:1/18:2)	784.5851	PC(36:3)[b]	
786.6008	PC(36:2)	(18:1/18:1) and (18:0/18:2)	786.6	PC(36:2)[a]	
788.6136	PC(36:1)[b]		788.6149	PC(36:1)[b]	
800.6155	PC(37:2)	(18:1/19:1)	800.6153	PC(37:2)	(18:1/19:1)
798.6003	PC(37:3)	(18:2/19:1)	798.601	PC(37:3)	(18:2/19:1)
796.5854	PC(37:4)	(19:1/18:3) and (18:2/19:2)	796.5842	PC(37:4)	(19:1/18:3) and (18:2/19:2)
794.5686	PC(37:5)	(19:2/18:3)	794.567	PC(37:5)[b]	
800.5215	PC(38:9)[b]		800.5212	PC(38:9)[b]	
802.5363	PC(38:8)[b]		802.5373	PC(38:8)[b]	
804.5522	PC(38:7)	(18:3/20:4)	804.5525	PC(38:7)[b]	
806.568	PC(38:6)	(18:3/20:3) and (18:2/20:4)	806.5671	PC(38:6)[a]	
808.5861	PC(38:5)	(20:2/18:3) and (18:2/20:3)	808.5825	PC(38:5)[a]	
810.6025	PC(38:4)	(20:1/18:3) and (18:2/20:2)	810.6027	PC(38:4)	(20:1/18:3) and (18:2/20:2)
812.6199	PC(38:3)	(20:0/18:3), (20:1/18:2) and (18:1/20:2)	812.6206	PC(38:3)	(20:0/18:3), (20:1/18:2) and (18:1/20:2)

	814.6341	PC(38:2)	(18:1/20:1), (20:0/18:2) and (19:1/19:1),	814.6342	PC(38:2)	(18:1/20:1), (20:0/18:2) and (19:1/19:1),
LPC	490.2939	LPC(16:3)[b]		–	–	
	492.3084	LPC(16:2)[a]		492.3082	LPC(16:2)[b]	
	494.3246	LPC(16:1)[b]		–	–	
	496.3405	LPC(16:0)	(16:0)	496.3407	LPC(16:0)	(16:0)
	518.3249	LPC(18:3)	(18:3)	518.3244	LPC(18:3)[a]	
	520.3406	**LPC(18:2)**	(18:2)	**520.3406**	**LPC(18:2)**	**(18:2)**
	522.3566	LPC(18:1)[b]		522.3571	LPC(18:1)[a]	
	524.3723	LPC(18:0)[a]		–	–	
	542.3226	LPC(20:5)[b]		–	–	
	544.3393	LPC(20:4)[b]		–	–	
PE	658.4427	PE(30:3)[c]		658.4428	PE(30:3)[c]	
	660.4626	PE(30:2)	(14:0/16:2), (14:1/16:1) and (15:1/15:1),	660.4578	PE(30:2)[c]	
	662.4767	PE(30:1)	(15:0/15:1) and (14:0/16:1)	–	–	
	664.4921	PE(30:0)	(15:0/15:0) and (14:0/16:0)	–	–	
	–	–		680.4261	PE(32:6)	(16:3/16:3)
	–	–		682.4459	PE(32:5)	(16:2/16:3)
	684.4594	PE(32:4)	(16:1/16:3) and (16:2/16:2)	684.4609	PE(32:4)	(16:1/16:3) and (16:2/16:2)
	686.4769	PE(32:3)	(16:0/16:3), (16:1/16:2) and (14:0/18:3)	686.4765	PE(32:3)	(16:0/16:3) and (16:1/16:2)
	688.4927	PE(32:2)	(16:0/16:2) and (16:1/16:1)	688.4927	PE(32:2)	(16:0/16:2)
	690.5079	PE(32:1)	(16:0/16:1) and (15:0/17:1)	690.5044	PE(32:1)	(16:0/16:1) and (14:0/18:1)
	700.493	PE(33:3)	(15:0/18:3) and (17:0/16:3)	700.4933	PE(33:3)	(17:0/16:3)
	708.4598	PE(34:6)	(16:3/18:3)	708.4592	PE(34:6)	(16:3/18:3)
	710.4758	PE(34:5)	(18:2/16:3) and (16:2/18:3)	710.4753	PE(34:5)	(18:2/16:3) and (16:2/18:3)
	712.4911	PE(34:4)	(16:2/18:2), (16:1/18:3) and (18:1/16:3)	712.4923	PE(34:4)	(16:2/18:2), (16:1/18:3) and (18:1/16:3)
	714.5077	PE(34:3)	(16:0/18:3), (16:1/18:2) and (18:1/16:2)	714.508	PE(34:3)	(16:0/18:3), (16:1/18:2) and (18:1/16:2)
	716.5233	**PE(34:2)**	**(16:0/18:2) and (16:1/18:1)**	**716.5237**	**PE(34:2)**	**(16:1/18:1) and (16:0/18:2)**
	718.5372	PE(34:1)	(16:0/18:1), (14:0/20:1) and (17:0/17:1)	718.5379	PE(34:1)	(16:0/18:1) and (14:0/20:1)
	730.5387	PE(35:2)	(17:0/18:2) and (17:1/18:1)	730.5397	PE(35:2)	(17:0/18:2) and (17:1/18:1)

	728.5244	PE(35:3)	(17:1/18:2) and (17:0/18:3)	728.5248	PE(35:3)	(17:1/18:2) and (17:0/18:3)
	726.5078	PE(35:4)	(17:1/18:3); (17:2/18:2) and (19:1/16:3)	726.5082	PE(35:4)	(17:1/18:3); (17:2/18:2) and (16:2/19:2)
	730.4429	PE(36:9)[c]		730.4419	PE(36:9)[c]	
	732.4573	PE(36:8)[c]		732.4577	PE(36:8)[c]	
	734.4779	PE(36:7)[c]		734.4739	PE(36:7)[c]	
	736.4918	PE(36:6)	(18:3/18:3)	736.4911	PE(36:6)	(18:3/18:3)
	738.5079	PE(36:5)	(18:2/18:3)	738.5069	PE(36:5)	(18:2/18:3)
	756.5182	PE(36:4(OH))[c]		756.5186	PE(36:4(OH))	(18:2/18:2-OH)
	740.523	PE(36:4)	(18:2/18:2) and (18:1/18:3)	740.5231	PE(36:4)	(18:2/18:2) and (18:1/18:3)
	742.5378	PE(36:3)	(18:1/18:2)	742.5369	PE(36:3)	(18:1/18:2)
	744.5552	PE(36:2)	(18:0/18:2) and (18:1/18:1)	744.5559	PE(36:2)	(18:0/18:2) and (18:1/18:1)
	752.5226	PE(37:5)	(19:2/18:3)	752.5228	PE(37:5)	(19:2/18:3)
	758.4733	PE(38:9)[c]		758.4731	PE(38:9)[c]	
	760.4886	PE(38:8)[c]		760.4887	PE(38:8)[c]	
	762.5045	PE(38:7)[a]		762.5053	PE(38:7)[c]	
LPE	452.2775	LPE(16:1)	(16:1)	–	–	
	454.293	LPE(16:0)	(16:0)	454.2931	LPE(16:0)	(16:0)
	476.2778	LPE(18:3)	(18:3)	–	–	
	478.2933	LPE(18:2)	(18:2)	**478.2936**	**LPE(18:2)**	**(18:2)**
	480.3093	**LPE(18:1)**	**(18:1)**	480.3091	LPE(18:1)	(18:1)
	500.2756	LPE(20:5)[a]		500.2757	LPE(20:5)[a]	
	502.2915	LPE(20:4)[a]		–	–	
Cer	554.5512	Cer(d35:0)[d]		554.5518	Cer(d35:0)	

C-Auto, *Chlorella vulgaris* cultivated under autotrophic conditions, C-Hetero, *Chlorella vulgaris* cultivated under heterotrophic conditions, PC, phosphatidylcholine, LPC, lysophosphatidylcholine, PE, phosphatidylethanolamine, LPE, lysophosphatidylethanolamine, Cer, ceramide.

[a] Lipid species identified by retention time and mass accuracy calculation

[b] Lipid species of PC and LPC identified by retention time, mass accuracy calculation, and typical product ion observed at *m/z* 184 in the HILIC-ESI-MS/MS spectrum of $[M+H]^+$ ion

[c] Lipid species of PE and LPE identified by retention time, mass accuracy calculation, and typical neutral loss of 141 in the HILIC-ESI-MS/MS spectrum of $[M+H]^+$ ion

[d] Lipid species of Cer identified by retention time, mass accuracy calculation, and typical product ion observed at *m/z* 264 in the HILIC-ESI-MS/MS spectrum of [M+H]$^+$ ion

* In phospholipids fatty acids (FA) with a lower acyl chain length are typically in the *sn*-1 position of the glycerol backbone and FA with a longer acyl chain length are typically in the *sn*-2 position [56–58].

Table S2.4. List of molecular ions and lipid species of phosphatidylglycerol (PG), phosphatidylinositol (PI) and inositolphosphoceramide (PI_Cer) identified by HILIC-ESI-MS in negative ion mode, as [M−H]$^-$ ions, in the total lipid extracts of *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions. When possible, the identification of the fatty acyl composition correspondent to each molecular ion was confirmed by the analysis of the LC-MS/MS spectra of each [M−H]$^-$ ion. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains, bold *m/z* values correspond to the most abundant molecular ions detected.

[M − H]$^-$	**Observed *m/z* of molecular ions in MS of the C-Auto**	**Lipid species (C:N) of the C-Auto**	**Fatty acyl chains observed in MS/MS of the C-Auto***	**Observed *m/z* of molecular ions in MS of the C-Hetero**	**Lipid species (C:N) of the C-Hetero**	**Fatty acyl chains observed in MS/MS of the C-Hetero***
PG	719.4884	PG(32:1)	(16:0/16:1) and (15:0/17:1)	719.4868	PG(32:1)[a]	
	721.5039	PG(32:0)	(16:0/16:0)	721.5043	PG(32:0)	(16:0/16:0)
	735.5199	PG(33:0)	(16:0/17:0)	735.5199	PG(33:0)	(16:0/17:0)
	741.4724	PG(34:4)	(16:2/18:2), (16:1/18:3) and (17:2/17:2)	741.47	PG(34:4)	(16:2/18:2), (16:1/18:3) and (18:1/16:3)
	743.4883	**PG(34:3)**	**(16:1/18:2) (16:0/18:3) and (17:0/17:3), (17:2/17:1)**	743.4885	PG(34:3)	(16:0/18:3)
	745.5041	**PG(34:2)[a]**		**745.5043**	**PG(34:2)**	**(16:0/18:2)**
	747.5186	PG(34:1)	(17:0/17:1) and (16:0/18:1)	**747.5198**	**PG(34:1)**	**(17:0/17:1) and (16:0/18:1)**
	749.5338	PG(34:0)	(16:0/18:0) and (17:0/17:0)	749.5316	PG(34:0)	(16:0/18:0) and (17:0/17:0)
	767.4878	PG(36:5)[a]		767.4875	PG(36:5)	(18:2/18:3)
	769.5023	PG(36:4)	(18:2/18:2) and (18:1/18:3)	769.5036	PG(36:4)	(18:2/18:2) and (18:1/18:3)

	771.5196	PG(36:3)	(18:0/18:3), (18:1/18:2) and (16:0/20:3)	771.5189	PG(36:3)	(18:1/18:2)
	773.5351	PG(36:2)	(18:1/18:1) and (18:0/18:2)	773.5344	PG(36:2)	(18:1/18:1) and (18:0/18:2)
	775.5498	PG(36:1)	(18:0/18:1) and (17:0/19:1)	775.5507	PG(36:1)	(18:0/18:1) and (17:0/19:1)
	777.5654	PG(36:0)	(18:0/18:0) and (17:0/19:0)	777.5644	PG(36:0)	(18:0/18:0) and (17:0/19:0)
	795.5183	PG(38:5)[a]		795.521	PG(38:5)[a]	
PI	–	–		805.4847	PI(32:2)	(14:1/18:1); (16:0/16:2) and (14:0/18:2)
	831.5034	PI(34:3)	(16:0/18:3), (16:1/18:2), (18:0/16:3) and (17:0/17:3)	831.5025	PI(34:3)	(16:0/18:3), (16:1/18:2), (18:0/16:3), (17:0/17:3) and (18:1/16:2)
	833.5139	**PI(34:2)**	**(16:0/18:2)**	**833.5198**	**PI(34:2)**	**(16:0/18:2)**
	835.5308	PI(34:1)[a]		835.5344	PI(34:1)[a]	
	847.5337	PI(35:2)[a]		847.5353	PI(35:2)	(17:0/18:2)
	855.5028	PI(36:5)[a]		855.5035	PI(36:5)[a]	
	–	–		857.5197	PI(36:4)	(18:2/18:2)
	–	–		859.5345	PI(36:3)	(18:1/18:2)
	–	–		861.5492	PI(36:2)	(18:0/18:2) and (18:1/18:1)
PI_Cer	812.5295	PI-Cer(t34:0(OH))	(t18:0/16:0(OH))	812.5271	PI-Cer(t34:0(OH))	(t18:0/16:0(OH))

C-Auto, *Chlorella vulgaris* cultivated under autotrophic conditions, C-Hetero, *Chlorella vulgaris* cultivated under heterotrophic conditions, PG, phosphatidylglycerol, PI, phosphatidylinositol, PI_Cer, inositolphosphoceramide.

[a] Lipid species identified by retention time and mass accuracy calculation.

* In phospholipids fatty acids (FA) with a lower acyl chain length are typically in the *sn*-1 position of the glycerol backbone and FA with a longer acyl chain length are typically in the *sn*-2 position [56–58].

Table S2.5. List of molecular ions and lipid species of sulfoquinovosyldiacylglycerol (SQDG) identified by HILIC-ESI-MS in negative ion mode, as [M−H]$^-$ ions, in the total lipid extracts of *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions. When possible, the identification of the fatty acyl composition correspondent to each molecular ion was confirmed by the analysis of the LC-MS/MS spectra of each [M−H]$^-$ ions. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains, bold *m/z* values correspond to the most abundant molecular ions detected.

[M − H]$^-$	Observed *m/z* of molecular ions in MS of the C-Auto	Lipid species (C:N) of the C-Auto	Fatty acyl chains observed in MS/MS of the C-Auto*	Observed *m/z* of molecular ions in MS of the C-Hetero	Lipid species (C:N) of the C-Hetero	Fatty acyl chains observed in MS/MS of the C-Hetero*
SQDG	–	–		765.4824	SQDG(30:0)^b	
	787.4692	SQDG(32:3)	(18:3/14:0)	787.47	SQDG(32:3)^b	
	789.4829	SQDG(32:2)^b		789.484	SQDG(32:2)^b	
	791.4975	SQDG(32:1)	(16:1/16:0)	791.4995	SQDG(32:1)^a	
	793.5151	**SQDG(32:0)**	**(16:0/16:0)**	**793.5157**	**SQDG(32:0)**	**(16:0/16:0)**
	811.4698	SQDG(34:5)^b		811.4683	SQDG(34:5)^b	
	813.4844	SQDG(34:4)^b		813.4843	SQDG(34:4)^b	
	815.5004	**SQDG(34:3)**	**(18:3/16:0)**	815.5003	SQDG(34:3)	(18:3/16:0)
	817.5149	SQDG(34:2)^a		817.5162	SQDG(34:2)	(18:2/16:0)
	819.5304	SQDG(34:1)^b		**819.5315**	**SQDG(34:1)^b**	
	821.5445	SQDG(34:0)^b		821.542	SQDG(34:0)^a	
	837.4851	SQDG(36:6)	(18:3/18:3)	–	–	
	839.5013	SQDG(36:5)^a		839.5	SQDG(36:5)^b	
	841.5162	SQDG(36:4)^a		841.5173	SQDG(36:4)	(18:2/18:2)
	843.5326	SQDG(36:3)^a		843.5323	SQDG(36:3)^a	

C-Auto, *Chlorella vulgaris* cultivated under autotrophic conditions, C-Hetero, *Chlorella vulgaris* cultivated under heterotrophic conditions, SQDG, sulfoquinovosyldiacylglycerol.

[a] Lipid species identified by retention time and mass accuracy calculation

[b] Lipid species of SQDG identified by retention time, mass accuracy calculation, and typical product ion observed at *m/z* 225 in the HILIC-ESI-MS/MS spectrum of [M−H]$^-$ ion

*In glycolipids, fatty acids (FA) with a lower acyl chain length are typically in the *sn*-2 position of glycerol backbone [59] and FA with a longer acyl chain length are typically in the *sn*-1 position [22].

Table S2.6. List of molecular ions and lipid species of monogalactosylmonoacylglycerol (MGMG), monogalactosyldiacylglycerol (MGDG), digalactosylmonoacylglycerol (DGMG) and digalactosyldiacylglycerol (DGDG) identified by HILIC-ESI-MS in positive ion mode, as [M+NH$_4$]$^+$ ions, in the total lipid extracts of *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions. When possible, the identification of the fatty acyl composition correspondent to each molecular ion was confirmed by the analysis of the LC-MS/MS spectra of each [M+NH$_4$]$^+$ ions. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains, bold *m/z* values correspond to the most abundant molecular ions detected.

[M + NH$_4$]$^+$	**Observed *m/z* of molecular ions in MS of the C-Auto**	**Lipid species (C:N) of the C-Auto**	**Fatty acyl chains observed in MS/MS of the C-Auto***	**Observed *m/z* of molecular ions in MS of the C-Hetero**	**Lipid species (C:N) of the C-Hetero**	**Fatty acyl chains observed in MS/MS of the C-Hetero***
MGMG	504.3176	MGMG(16:3)[a]		504.3181	**MGMG(16:3)[a]**	
	506.3329	MGMG(16:2)[a]		506.3327	MGMG(16:2)	(16:2)
	–	–		510.3635	MGMG(16:0)	(16:0)
	532.3491	**MGMG(18:3)**	**(18:3)**	–	–	
	534.3646	MGMG(18:2)	(18:2)	534.3652	MGMG (18:2)	(18:2)
MGDG	736.5008	MGDG(32:6)	(16:3/16:3) and (17:3/15:3)	–	–	
	738.5165	MGDG(32:5)	(16:3/16:2) and (16:4/16:1)	738.5176	MGDG(32:5)[a]	
	740.5328	MGDG(32:4)	(16:2/16:2) and (16:3/16:1)	740.534	MGDG(32:4)	(16:2/16:2) and (16:3/16:1)
	742.5474	MGDG(32:3)[a]		742.5496	MGDG(32:3)[a]	
	744.5615	MGDG(32:2)	(16:1/16:1) and (16:2/16:0)	744.5629	MGDG(32:2)	(16:1/16:1) and (16:2/16:0)
	–	–		762.5182	MGDG(34:7)[a]	
	764.5327	**MGDG(34:6)**	**(18:3/16:3), (18:2/16:4) and (18:4/16:2)**	764.5317	MGDG(34:6)	(18:3/16:3), (18:2/16:4) and (18:4/16:2)
	766.5463	**MGDG(34:5)**	**(18:3/16:2) and (18:2/16:3)**	766.5507	**MGDG(34:5)**	**(18:3/16:2) and (18:2/16:3)**
	768.5626	MGDG(34:4)	(18:1/16:3), (18:2/16:2) and (18:3/16:1)	768.5637	**MGDG(34:4)**	**(18:1/16:3); (18:2/16:2) and (18:3/16:1)**

	770.577	MGDG(34:3)	(18:3/16:0), (18:2/16:1) and (18:1/16:2)	770.5779	MGDG(34:3)	(18:3/16:0), (18:2/16:1) and (18:1/16:2)
	772.5936	MGDG(34:2)	(18:1/16:1), (18:2/16:0), (17:2/17:0) and (17:1/17:1)	772.5946	MGDG(34:2)	(18:1/16:1) and (18:2/16:0)
	774.6097	MGDG(34:1)	(18:1/16:0)	774.6099	MGDG(34:1)	(18:1/16:0) and (18:0/16:1)
	776.6242	MGDG(34:0)[a]		776.6211	MGDG(34:0)	(18:0/16:0)
	788.624	MGDG(35:1)	(18:1/17:0)	788.6256	MGDG(35:1)	(18:1/17:0)
	792.5632	MGDG(36:6)	(18:3/18:3)	792.5652	MGDG(36:6)	(18:3/18:3)
	794.577	MGDG(36:5)	(18:3/18:2)	794.5802	MGDG(36:5)	(18:3/18:2)
	796.5933	MGDG(36:4)	(18:3/18:1), (18:2/18:2) and (20:1/16:3)	796.5946	MGDG(36:4)	(18:3/18:1) and (18:2/18:2)
	798.607	MGDG(36:3)	(18:3/18:0) and (18:2/18:1)	798.6082	MGDG(36:3)	(18:3/18:0) and (18:2/18:1)
	800.6242	MGDG(36:2)[a]		800.6229	MGDG(36:2)[a]	
DGMG	672.417	DGMG(16:0)	(16:0)	672.4175	DGMG(16:0)	(16:0)
DGDG	854.584	DGDG(28:0)[a]		854.5815	DGDG(28:0)[a]	
	900.5699	DGDG(32:5)	(16:3/16:2)	–	–	
	902.5844	DGDG(32:4)	(16:3/16:1) and (16:2/16:2)	–	–	
	904.6015	DGDG(32:3)	(16:3/16:0), (16:2/16:1) and (18:3/14:0)	904.6015	DGDG(32:3)	(16:3/16:0), (16:2/16:1) and (18:3/14:0)
	906.6153	DGDG(32:2)[a]		906.616	DGDG(32:2)	(16:2/16:0) and (18:2/14:0)
	908.6298	DGDG(32:1)	(16:1/16:0)	908.6285	DGDG(32:1)[a]	
	910.6469	DGDG(32:0)	(16:0/16:0)	910.648	DGDG(32:0)	(16:0/16:0)
	926.5852	**DGDG(34:6)**	**(18:3/16:3)**	926.5859	DGDG(34:6)	(18:3/16:3)
	928.5989	**DGDG(34:5)**	**(18:3/16:2) and (18:2/16:3)**	928.601	DGDG(34:5)	(18:3/16:2) and (18:2/16:3)

930.6162	DGDG(34:4)	(18:1/16:3), (18:2/16:2) and (18:3/16:1)	930.6162	DGDG(34:4)	(18:1/16:3); (18:2/16:2) and (18:3/16:1)
932.6305	DGDG(34:3)	(18:3/16:0), (18:2/16:1) and (18:1/16:2)	932.6299	DGDG(34:3)	(18:3/16:0), (18:2/16:1) and (18:1/16:2)
934.6471	DGDG(34:2)	(18:2/16:0), (18:1/16:1) and (18:0/16:2)	934.6477	**DGDG(34:2)**	**(18:2/16:0) and (18:1/16:1)**
936.6609	DGDG(34:1)	(18:1/16:0)	936.6624	**DGDG(34:1)**	**(18:1/16:0) and (18:0/16:1)**
950.6759	DGDG(35:1)[a]		950.6782	DGDG(35:1)	(18:1/17:0) and (19:1/16:0)
948.6625	DGDG(35:2)[a]		948.6638	DGDG(35:2)	(18:2/17:0)
946.6484	DGDG(35:3)	(18:3/17:0) and (18:2/17:1)	946.6485	DGDG(35:3)	(18:3/17:0) and (18:2/17:1)
952.601	DGDG(36:7)[a]		952.6024	DGDG(36:7)[a]	
954.6173	DGDG(36:6)	(18:3/18:3)	954.6198	DGDG(36:6)	(18:3/18:3)
956.6315	DGDG(36:5)	(18:3/18:2)	956.633	DGDG(36:5)	(18:3/18:2)
958.6458	DGDG(36:4)[a]		958.6476	DGDG(36:4)	(18:3/18:1) and (18:2/18:2)
960.6593	DGDG(36:3)[a]		960.6601	DGDG(36:3)	(18:2/18:1) and (18:3/18:0)
–	–		962.6779	DGDG(36:2)	(18:2/18:0)

C-Auto, *Chlorella vulgaris* cultivated under autotrophic conditions, C-Hetero, *Chlorella vulgaris* cultivated under heterotrophic conditions, DGDG, digalactosyldiacylglycerol, DGMG, digalactosylmonoacylglycerol, MGDG, monogalactosyldiacylglycerol, MGMG, monogalactosylmonoacylglycerol.

[a] Lipid species identified by retention time and mass accuracy calculation.

*In glycolipids, fatty acids (FA) with a lower acyl chain length are typically in the *sn*-2 position of glycerol backbone [59] and FA with a longer acyl chain length are typically in the *sn*-1 position [22].

Table S2.7. Results of the Wilcoxon rank-sum test of the 157 lipid species which were detected under the two conditions. Bold indicates the top 25 q values ($q < 0.05$).

	F.value	**pvalue**	**fdr**
DGDG.28.0.	**0**	**0.007937**	**0.024115**
DGDG.32.0.	**0**	**0.007937**	**0.024115**
DGDG.32.3.	**25**	**0.007937**	**0.024115**
DGDG.34.1.	**0**	**0.007937**	**0.024115**
DGDG.34.2.	**0**	**0.007937**	**0.024115**
DGDG.34.3.	**0**	**0.007937**	**0.024115**
DGDG.34.4.	**0**	**0.007937**	**0.024115**
DGDG.34.6.	**25**	**0.007937**	**0.024115**
DGDG.35.1.	**0**	**0.007937**	**0.024115**
DGDG.35.2.	**0**	**0.007937**	**0.024115**
DGDG.36.3.	**0**	**0.007937**	**0.024115**
DGDG.36.7.	**25**	**0.007937**	**0.024115**
LPC.18.1.	**25**	**0.007937**	**0.024115**
LPC.18.3.	**25**	**0.007937**	**0.024115**
LPE.18.1.	**25**	**0.007937**	**0.024115**
MGDG.32.4.	**25**	**0.007937**	**0.024115**
MGDG.32.5.	**25**	**0.007937**	**0.024115**
MGDG.34.0.	**0**	**0.007937**	**0.024115**
MGDG.34.1.	**0**	**0.007937**	**0.024115**
MGDG.34.2.	**0**	**0.007937**	**0.024115**
MGDG.34.6.	**25**	**0.007937**	**0.024115**
MGDG.35.1.	**0**	**0.007937**	**0.024115**
MGDG.36.2.	**0**	**0.007937**	**0.024115**
MGDG.36.5.	**25**	**0.007937**	**0.024115**
MGDG.36.6.	**25**	**0.007937**	**0.024115**
MGMG.16.2.	0	0.007937	0.024115
MGMG.16.3.	0	0.007937	0.024115
PC.30.3.	25	0.007937	0.024115
PC.32.5.	25	0.007937	0.024115
PC.32.6.	25	0.007937	0.024115
PC.34.6.	25	0.007937	0.024115
PC.34.8.	25	0.007937	0.024115
PC.35.4.	25	0.007937	0.024115
PC.36.6.	25	0.007937	0.024115
PC.36.9.	25	0.007937	0.024115
PC.38.9.	25	0.007937	0.024115
PE.30.2.	25	0.007937	0.024115
PE.30.3.	25	0.007937	0.024115
PE.38.7.	0	0.007937	0.024115
PG.32.1.	25	0.007937	0.024115
PG.34.3.	25	0.007937	0.024115
PG.34.4.	25	0.007937	0.024115

PG.36.2.	25	0.007937	0.024115
PI.34.1.	0	0.007937	0.024115
PI.34.2.	0	0.007937	0.024115
SQDG.32.1.	25	0.007937	0.024115
SQDG.32.3.	25	0.007937	0.024115
SQDG.34.3.	25	0.007937	0.024115
SQDG.34.4.	25	0.007937	0.024115
SQDG.34.5.	25	0.007937	0.024115
SQDG.36.3.	0	0.007937	0.024115
SQDG.36.5.	25	0.007937	0.024115
DGDG.34.5.	1	0.015873	0.034355
DGDG.36.4.	1	0.015873	0.034355
MGDG.34.3.	1	0.015873	0.034355
MGDG.36.3.	1	0.015873	0.034355
PC.32.2.	1	0.015873	0.034355
PC.34.1.	1	0.015873	0.034355
PC.34.7.	24	0.015873	0.034355
PC.35.1.	1	0.015873	0.034355
PC.36.3.	1	0.015873	0.034355
PE.32.1.	24	0.015873	0.034355
PE.32.2.	1	0.015873	0.034355
PE.34.1.	1	0.015873	0.034355
PE.35.4.	24	0.015873	0.034355
PE.36.3.	1	0.015873	0.034355
PE.36.4.	1	0.015873	0.034355
PE.36.6.	24	0.015873	0.034355
PE.38.9.	24	0.015873	0.034355
PG.34.1.	1	0.015873	0.034355
PI.35.2.	1	0.015873	0.034355
SQDG.34.1.	1	0.015873	0.034355
DGDG.36.6.	23	0.031746	0.065141
LPC.18.2.	23	0.031746	0.065141
PC.38.6.	2	0.031746	0.065141
SQDG.32.2.	23	0.031746	0.065141
Cer.d35.0.	3	0.055556	0.100894
DGDG.32.2.	3	0.055556	0.100894
MGDG.34.4.	3	0.055556	0.100894
MGDG.36.4.	3	0.055556	0.100894
PC.32.4.	22	0.055556	0.100894
PC.33.3.	22	0.055556	0.100894
PC.36.4.	3	0.055556	0.100894
PE.34.2.	3	0.055556	0.100894
PG.38.5.	22	0.055556	0.100894
PI_Cer.t18.0.16.0.2OH..	22	0.055556	0.100894
DGMG.16.0.	4	0.095238	0.13933
LPC.16.2.	21	0.095238	0.13933

LPE.16.0.	21	0.095238	0.13933
PC.34.2.	4	0.095238	0.13933
PC.35.3.	21	0.095238	0.13933
PC.36.1.	4	0.095238	0.13933
PC.36.2.	4	0.095238	0.13933
PC.36.4.OH..	21	0.095238	0.13933
PC.38.3.	4	0.095238	0.13933
PC.38.7.	4	0.095238	0.13933
PC.38.8.	21	0.095238	0.13933
PE.32.3.	4	0.095238	0.13933
PE.32.4.	21	0.095238	0.13933
PE.33.3.	21	0.095238	0.13933
PE.34.4.	4	0.095238	0.13933
PE.34.5.	4	0.095238	0.13933
PE.35.3.	21	0.095238	0.13933
PE.36.7.	4	0.095238	0.13933
PE.36.8.	4	0.095238	0.13933
PG.36.0.	21	0.095238	0.13933
SQDG.34.2.	4	0.095238	0.13933
LPC.16.0.	20	0.150794	0.20191
MGDG.32.3.	20	0.150794	0.20191
PC.34.3.	20	0.150794	0.20191
PC.35.2.	5	0.150794	0.20191
PC.36.8.	20	0.150794	0.20191
PC.37.3.	5	0.150794	0.20191
PC.37.4.	20	0.150794	0.20191
PC.38.5.	5	0.150794	0.20191
PG.34.0.	5	0.150794	0.20191
PG.36.4.	20	0.150794	0.20191
MGDG.32.2.	6	0.222222	0.287796
MGMG.18.2.	19	0.222222	0.287796
PE.38.8.	6	0.222222	0.287796
PI.34.3.	19	0.222222	0.287796
PC.34.4.	7	0.309524	0.367705
PE.34.3.	18	0.309524	0.367705
PE.34.6.	18	0.309524	0.367705
PE.35.2.	7	0.309524	0.367705
PE.36.5.	7	0.309524	0.367705
PE.36.9.	18	0.309524	0.367705
PG.32.0.	7	0.309524	0.367705
PG.33.0.	7	0.309524	0.367705
PG.34.2.	18	0.309524	0.367705
SQDG.32.0.	7	0.309524	0.367705
SQDG.36.4.	7	0.309524	0.367705
DGDG.35.3.	17	0.420635	0.47135
LPE.18.2.	8	0.420635	0.47135

LPE.20.5.	8	0.420635	0.47135
PC.32.0.	8	0.420635	0.47135
PC.38.2.	8	0.420635	0.47135
PE.36.2.	8	0.420635	0.47135
PE.37.5.	8	0.420635	0.47135
PG.36.3.	17	0.420635	0.47135
DGDG.32.1.	9	0.547619	0.580697
MGDG.34.5.	16	0.547619	0.580697
PC.36.7.	9	0.547619	0.580697
PC.37.2.	9	0.547619	0.580697
PC.38.4.	9	0.547619	0.580697
PE.36.4.OH..	16	0.547619	0.580697
PG.36.1.	16	0.547619	0.580697
PI.36.5.	16	0.547619	0.580697
DGDG.36.5.	15	0.690476	0.70384
PC.32.3.	10	0.690476	0.70384
PC.33.2.	10	0.690476	0.70384
PC.34.5.	15	0.690476	0.70384
PC.36.5.	15	0.690476	0.70384
SQDG.34.0.	15	0.690476	0.70384
PC.37.5.	11	0.84127	0.846628
PG.36.5.	14	0.84127	0.846628
PC.32.1.	13	1	1

PC, phosphatidylcholine, LPC, lysophosphatidylcholine, PE, phosphatidylethanolamine, LPE, lysophosphatidylethanolamine, PG, phosphatidylglycerol, PI, phosphatidylinositol, DGDG, digalactosyldiacylglycerol, DGMG, digalactosylmonoacylglycerol, MGDG, monogalactosyldiacylglycerol, MGMG, monogalactosylmonoacylglycerol, SQDG, sulfoquinovosyldiacylglycerol, Cer, Ceramide, PI_Cer, inositolphosphoceramide.

Supplementary material of Chapter 3

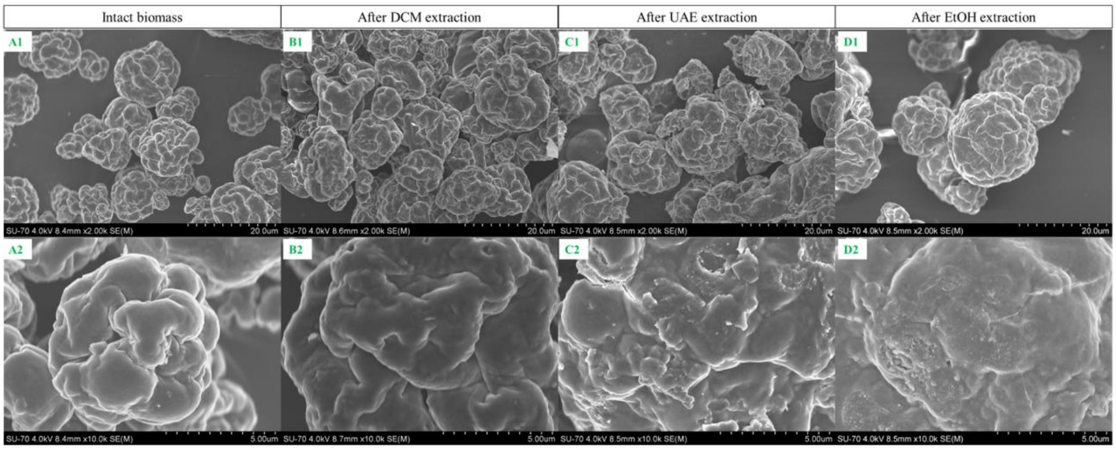


Figure S3.1. Scanning electron micrographs of the cell surfaces of *Chlorella vulgaris* grown under autotrophic conditions. (**A1**-**A2**) Cells before extraction, (**B1**-**B2**) cells after extraction with dichloromethane/methanol (DCM), (**C1**-**C2**) cells after ethanol extraction assisted by an ultrasound probe (UAE), (**D1**-**D2**) cells after ethanol extraction (EtOH). C-Auto cells presented a non-well-defined shape. The DCM extraction did not significantly change the cell surface and shape. The ethanol and UAE extraction maintained the general initial form of the cells but enhanced their surface roughness (surface attack).

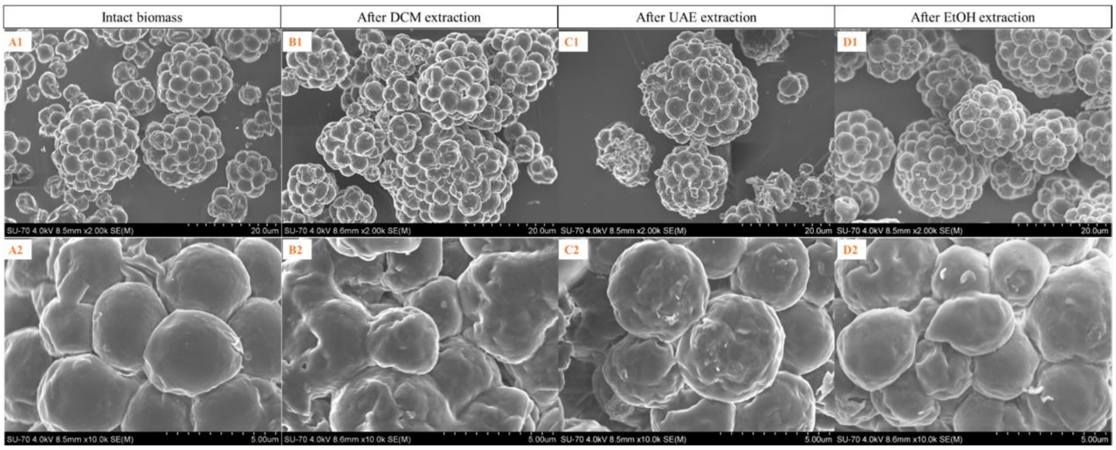


Figure S3.2. Scanning electron micrographs of the cell surfaces of *Chlorella vulgaris* grown under heterotrophic conditions. (**A1**-**A2**) Cells before extraction, (**B1**-**B2**) cells after extraction with dichloromethane/methanol (DCM), (**C1**-**C2**) cells after ethanol extraction assisted by an ultrasound probe (UAE), (**D1**-**D2**) cells after ethanol extraction (EtOH). C-Hetero cells presented an initial round-shaped form, which was maintained after ethanol and UAE extractions. UAE extraction increased the surface roughness of the cells. The DCM extractions led to a specific loss of the round shape of the cells.

Table S3.1. Exact mass measurements of the molecular ions identified by HILIC−ESI−MS in the DCM, EtOH and UAE extracts from *Chlorella vulgaris* cultivated under autotrophic conditions (C-Auto). C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains.

Lipid species (C:N)	**Formula**	**Calculated *m/z***	**Observed *m/z* in MS of the DCM extracts**	**Error (ppm)**	**Observed *m/z* in MS of the UAE extracts**	**Error (ppm)**	**Observed *m/z* in MS of the Ethanol extracts**	**Error (ppm)**
PC identified as $[M+H]^+$								
PC(30:3)	C38H71NO8P	700.4917	700.4893	**-3.4718**	700.4900	**-2.4725**	700.4918	**0.0971**
PC(32:6)	C40H69NO8P	722.4761	722.4758	**-0.3903**	722.4765	**0.5786**	722.4782	**2.9316**
PC(32:5)	C40H71NO8P	724.4917	724.4914	**-0.4583**	724.4923	**0.7840**	724.4940	**3.1305**
PC(32:4)	C40H73NO8P	726.5074	726.5069	**-0.6634**	726.5079	**0.7130**	726.5094	**2.7777**
PC(32:3)	C40H75NO8P	728.5230	728.5225	**-0.7302**	728.5231	**0.0933**	728.5249	**2.5641**
PC(32:2)	C40H77NO8P	730.5387	730.5379	**-1.0704**	730.5387	**0.0246**	730.5406	**2.6255**
PC(32:1)	C40H79NO8P	732.5543	732.5528	**-2.0899**	732.5536	**-0.9979**	732.5555	**1.5958**
PC(32:0)	C40H81NO8P	734.5700	734.5680	**-2.6982**	734.5682	**-2.4259**	734.5700	**0.0245**
PC(33:2)	C41H79NO8P	744.5543	744.5536	**-0.9818**	744.5545	**0.2270**	744.5564	**2.7788**
PC(33:3)	C41H77NO8P	742.5387	742.5383	**-0.5145**	742.5391	**0.5629**	742.5410	**3.1217**
PC(34:8)	C42H69NO8P	746.4761	746.4734	**-3.5929**	746.4739	**-2.9231**	746.4761	**0.0241**
PC(34:7)	C42H71NO8P	748.4917	748.4895	**-2.9820**	748.4905	**-1.6460**	748.4927	**1.2933**
PC(34:6)	C42H73NO8P	750.5074	750.5067	**-0.9087**	750.5075	**0.1572**	750.5094	**2.6888**
PC(34:5(OH))	C42H75NO9P	768.5179	768.5162	**-2.2732**	768.5168	**-1.4925**	768.5188	**1.1099**
PC(34:5)	C42H75NO8P	752.5230	752.5218	**-1.6372**	752.5227	**-0.4412**	752.5246	**2.0837**
PC(34:4)	C42H77NO8P	754.5387	754.5376	**-1.4340**	754.5385	**-0.2412**	754.5404	**2.2769**
PC(34:3)	C42H79NO8P	756.5543	756.5534	**-1.2306**	756.5541	**-0.3053**	756.5560	**2.2061**
PC(34:2)	C42H81NO8P	758.5700	758.5688	**-1.5582**	758.5696	**-0.5036**	758.5715	**2.0011**
PC(34:1)	C42H83NO8P	760.5856	760.5830	**-3.4605**	760.5835	**-2.8031**	760.5857	**0.0894**
PC(35:1)	C43H85NO8P	774.6013	774.5990	**-2.9460**	774.5998	**-1.9132**	774.6019	**0.7978**

PC(35:2)	C43H83NO8P	772.5856	772.5846	**-1.3358**	772.5854	**-0.3003**	772.5875	**2.4179**
PC(35:3)	C43H81NO8P	770.5700	770.5690	**-1.2744**	770.5696	**-0.4957**	770.5717	**2.2295**
PC(35:4)	C43H79NO8P	768.5543	768.5533	**-1.3415**	768.5540	**-0.4307**	768.5561	**2.3017**
PC(36:9)	C44H71NO8P	772.4917	772.4888	**-3.7955**	772.4897	**-2.6304**	772.4917	**-0.0414**
PC(36:8)	C44H73NO8P	774.5074	774.5039	**-4.4958**	774.5047	**-3.4628**	774.5067	**-0.8806**
PC(36:7)	C44H75NO8P	776.5230	776.5192	**-4.9348**	776.5206	**-3.1319**	776.5227	**-0.4275**
PC(36:6)	C44H77NO8P	778.5387	778.5375	**-1.5182**	778.5382	**-0.6191**	778.5403	**2.0783**
PC(36:5)	C44H79NO8P	780.5543	780.5527	**-2.0895**	780.5536	**-0.9365**	780.5557	**1.7539**
PC(36:4(OH))	C44H81NO9P	798.5649	798.5621	**-3.5025**	798.5613	**-4.5043**	798.5662	**1.6317**
PC(36:4)	C44H81NO8P	782.5700	782.5687	**-1.6382**	782.5694	**-0.7437**	782.5716	**2.0675**
PC(36:3)	C44H83NO8P	784.5856	784.5837	**-2.4624**	784.5842	**-1.8252**	784.5862	**0.7239**
PC(36:2)	C44H85NO8P	786.6013	786.5997	**-2.0112**	786.6002	**-1.3755**	786.6025	**1.5484**
PC(36:1)	C44H87NO8P	788.6169	788.6133	**-4.6055**	788.6146	**-2.9571**	788.6158	**-1.4354**
PC(37:2)	C45H87NO8P	800.6169	800.6156	**-1.6637**	800.6161	**-1.0392**	800.6181	**1.4589**
PC(37:3)	C45H85NO8P	798.6013	798.5999	**-1.7305**	798.6007	**-0.7288**	798.6030	**2.1513**
PC(37:4)	C45H83NO8P	796.5856	796.5837	**-2.4254**	796.5855	**-0.1657**	796.5876	**2.4705**
PC(37:5)	C45H81NO8P	794.5700	794.5676	**-2.9978**	794.5716	**2.0363**	794.5727	**3.4207**
PC(38:9)	C46H75NO8P	800.5230	800.5193	**-4.6620**	800.5203	**-3.4128**	800.5223	**-0.9144**
PC(38:8)	C46H77NO8P	802.5387	802.5345	**-5.2110**	802.5354	**-4.0895**	802.5374	**-1.5974**
PC(38:7)	C46H79NO8P	804.5543	804.5507	**-4.5131**	804.5514	**-3.6430**	804.5535	**-1.0329**
PC(38:6)	C46H81NO8P	806.5700	806.5657	**-5.3089**	806.5664	**-4.4410**	806.5688	**-1.4655**
PC(38:5)	C46H83NO8P	808.5856	808.5821	**-4.3681**	808.5852	**-0.5343**	808.5874	**2.1865**
PC(38:4)	C46H85NO8P	810.6013	810.6001	**-1.4582**	810.6000	**-1.5815**	810.6012	**-0.1012**
PC(38:3)	C46H87NO8P	812.6169	812.6161	**-1.0239**	812.6165	**-0.5316**	812.6182	**1.5604**
PC(38:2)	C46H89NO8P	814.6326	814.6320	**-0.7144**	814.6312	**-1.6965**	814.6300	**-3.1695**
LPC identified as $[M+H]^+$								
LPC(16:3)	C24H45NO7P	490.2934	490.2928	**-1.1565**	490.2930	**-0.7485**	490.2944	**2.1069**
LPC(16:2)	C24H47NO7P	492.3090	492.3087	**-0.6439**	492.3075	**-3.0814**	492.3096	**1.1842**

LPC(16:1)	C24H49NO7P	494.3247	494.3244	**-0.5401**	494.3245	**-0.3378**	494.3263	**3.3035**
LPC(16:0)	C24H51NO7P	496.3403	496.3398	**-1.0396**	496.3402	**-0.2337**	496.3417	**2.7884**
LPC(18:3)	C26H49NO7P	518.3247	518.3244	**-0.5151**	518.3245	**-0.3222**	518.3261	**2.7647**
LPC(18:2)	C26H51NO7P	520.3403	520.3403	**-0.0307**	520.3404	**0.1614**	520.3421	**3.4285**
LPC(18:1)	C26H53NO7P	522.3560	522.3563	**0.6375**	522.3564	**0.8289**	522.3578	**3.5091**
LPC(18:0)	C26H55NO7P	524.3716	524.3721	**0.9211**	524.3728	**2.2560**	524.3739	**4.3538**
LPC(20:5)	C28H49NO7P	542.3247	542.3223	**-4.3645**	542.3228	**-3.4426**	542.3247	**0.0608**
LPC(20:4)	C28H51NO7P	544.3403	544.3395	**-1.4991**	544.3396	**-1.3154**	544.3416	**2.3588**
PE identified as $[M+H]^+$								
PE(30:3)	C35H65NO8P	658.4448	658.4425	**-3.4657**	658.4427	**-3.1620**	658.4444	**-0.5802**
PE(30:2)	C35H67NO8P	660.4604	660.4587	**-2.6224**	660.4585	**-2.9252**	660.4613	**1.3142**
PE(30:1)	C35H69NO8P	662.4761	662.4760	**-0.1238**	662.4762	**0.1781**	662.4777	**2.4424**
PE(30:0)	C35H71NO8P	664.4917	664.4921	**0.5538**	664.4926	**1.3063**	664.4937	**2.9617**
PE(32:6)	C37H63NO8P	680.4291	_	_	_	_	_	_
PE(32:5)	C37H65NO8P	682.4448	_	_	_	_	_	_
PE(32:4)	C37H67NO8P	684.4604	684.4597	**-1.0695**	684.4602	**-0.3390**	684.4613	**1.2682**
PE(32:3)	C37H69NO8P	686.4761	686.4760	**-0.1195**	686.4761	**0.0262**	686.4778	**2.5026**
PE(32:2)	C37H71O8NP	688.4917	688.4917	**-0.0465**	688.4921	**0.5345**	688.4938	**3.0037**
PE(32:1)	C37H73NO8P	690.5074	690.5073	**-0.1188**	690.5077	**0.4605**	690.5091	**2.4880**
PE(33:3)	C38H71NO8P	700.4917	700.4919	**0.2398**	700.4922	**0.6681**	700.4936	**2.6667**
PE(34:6)	C39H67NO8P	708.4604	708.4593	**-1.5978**	708.4638	**4.7540**	708.4620	**2.2132**
PE(34:5)	C39H69NO8P	710.4761	710.4747	**-1.9452**	710.4752	**-1.2414**	710.4764	**0.4476**
PE(34:4)	C39H71NO8P	712.4917	712.4902	**-2.1502**	712.4907	**-1.4484**	712.4920	**0.3761**
PE(34:3)	C39H73O8NP	714.5074	714.5071	**-0.3947**	714.5079	**0.7250**	714.5094	**2.8243**
PE(34:2)	C39H75NO8P	716.5230	716.5225	**-0.7425**	716.5231	**0.0949**	716.5249	**2.6070**
PE(34:1)	C39H77NO8P	718.5387	718.5357	**-4.1501**	718.5363	**-3.3151**	718.5375	**-1.6450**
PE(35:2)	C40H77NO8P	730.5387	730.5384	**-0.3860**	730.5385	**-0.2491**	730.5402	**2.0779**
PE(35:3)	C40H75NO8P	728.5230	728.5227	**-0.4557**	728.5231	**0.0933**	728.5249	**2.5641**

PE(35:4)	C40H73NO8P	726.5074	726.5054	**-2.7281**	726.5057	**-2.3152**	726.5071	**-0.3882**
PE(36:9)	C41H65NO8P	730.4448	730.4414	**-4.6301**	730.4421	**-3.6717**	730.4434	**-1.8920**
PE(36:8)	C41H67NO8P	732.4604	732.4570	**-4.6856**	732.4572	**-4.4125**	732.4588	**-2.2281**
PE(36:7)	C41H69NO8P	734.4761	734.4729	**-4.3323**	734.4736	**-3.3793**	734.4748	**-1.7455**
PE(36:6)	C41H71NO8P	736.4917	736.4907	**-1.4012**	736.4910	**-0.9939**	736.4928	**1.4501**
PE(36:5)	C41H73O8NP	738.5074	738.5061	**-1.7359**	738.5069	**-0.6527**	738.5081	**0.9722**
PE(36:4(OH))	C41H75NO9P	756.5179	756.5183	**0.4666**	756.5175	**-0.5909**	756.5198	**2.4494**
PE(36:4)	C41H75NO8P	740.5230	740.5225	**-0.7184**	740.5228	**-0.3133**	740.5244	**1.8473**
PE(36:3)	C41H77NO8P	742.5387	742.5377	**-1.3225**	742.5376	**-1.4572**	742.5397	**1.3710**
PE(36:2)	C41H79O8NP	744.5543	744.5541	**-0.3103**	744.5549	**0.7642**	744.5568	**3.3161**
PE(37:5)	C42H75NO8P	752.5230	752.5208	**-2.9660**	752.5213	**-2.3016**	752.5228	**-0.3083**
PE(38:9)	C43H69NO8P	758.4761	758.4722	**-5.1182**	758.4725	**-4.7226**	758.4744	**-2.2176**
PE(38:8)	C43H71NO8P	760.4917	760.4881	**-4.7759**	760.4887	**-3.9869**	760.4901	**-2.1460**
PE(38:7)	C43H73NO8P	762.5074	762.5043	**-4.0419**	762.5046	**-3.6485**	762.5065	**-1.1567**
LPE identified as $[M+H]^+$								
LPE(16:1)	C21H43NO7P	452.2777	452.2772	**-1.1431**	452.2778	**0.1835**	452.2788	**2.3945**
LPE(16:0)	C21H45NO7P	454.2934	454.2935	**0.2928**	454.2942	**1.8336**	454.2949	**3.3745**
LPE(18:3)	C23H43NO7P	476.2777	476.2771	**-1.2955**	476.2774	**-0.6656**	476.2788	**2.2739**
LPE(18:2)	C23H45NO7P	478.2934	478.2933	**-0.1401**	478.2944	**2.1598**	478.2952	**3.8324**
LPE(18:1)	C23H47NO7P	480.3090	480.3089	**-0.2436**	480.3091	**0.1728**	480.3104	**2.8794**
LPE(20:5)	C25H43NO7P	500.2777	500.2751	**-5.2311**	500.2757	**-4.0318**	500.2771	**-1.2333**
LPE(20:4)	C25H45NO7P	502.2934	502.2913	**-4.1151**	502.2919	**-2.9206**	502.2930	**-0.7306**
PG identified as $[M-H]^-$								
PG(32:1)	C38H72O10P	719.4863	719.4866	**0.4170**	719.4871	**1.1119**	719.4892	**4.0307**
PG(32:0)	C38H74O10P	721.5020	721.5021	**0.1386**	721.5023	**0.4158**	721.5045	**3.4650**
PG(33:0)	C39H76O10P	735.5176	735.5173	**-0.4256**	735.5180	**0.5262**	735.5203	**3.6532**
PG(34:4)	C40H70O10P	741.4707	741.4706	**-0.1349**	741.4712	**0.6743**	741.4735	**3.7763**
PG(34:3)	C40H72O10P	743.4863	743.4861	**-0.2690**	743.4864	**0.1345**	743.4887	**3.2280**

PG(34:2)	C40H74O10P	745.5020	745.5016	**-0.5366**	745.5022	**0.2683**	745.5041	**2.8169**
PG(34:1)	C40H76O10P	747.5176	747.5159	**-2.2742**	747.5164	**-1.6053**	747.5182	**0.8027**
PG(34:0)	C40H78O10P	749.5333	749.5298	**-4.6696**	749.5304	**-3.8691**	749.5328	**-0.6671**
PG(36:5)	C42H72O10P	767.4863	767.4841	**-2.8665**	767.4850	**-1.6938**	767.4861	**-0.2606**
PG(36:4)	C42H74O10P	769.5020	769.5011	**-1.1696**	769.5012	**-1.0396**	769.5035	**1.9493**
PG(36:3)	C42H76O10P	771.5176	771.5183	**0.9073**	771.5164	**-1.5554**	771.5195	**2.4627**
PG(36:2)	C42H78O10P	773.5333	773.5322	**-1.4220**	773.5320	**-1.6806**	773.5344	**1.4220**
PG(36:1)	C42H80O10P	775.5489	775.5454	**-4.5129**	775.5462	**-3.4814**	775.5496	**0.9026**
PG(36:0)	C42H82O10P	777.5646	777.5623	**-2.9580**	777.5627	**-2.4435**	777.5653	**0.9002**
PG(38:5)	C44H76O10P	795.5176	795.5204	**3.5197**	795.5203	**3.3940**	795.5217	**5.1539**
PI identified as $[M-H]^-$								
PI(32:2)	C41H74O13P	805.4867	_	_	_	_	_	_
PI(34:3)	C43H76O13P	831.5024	831.5029	**0.6013**	831.5038	**1.6837**	831.5062	**4.5700**
PI(34:2)	C43H78O13P	833.5180	833.5165	**-1.7996**	833.5168	**-1.4397**	833.5187	**0.8398**
PI(34:1)	C43H80O13P	835.5337	835.5299	**-4.5480**	835.5301	**-4.3086**	835.5324	**-1.5559**
PI(35:2)	C44H80O13P	847.5337	847.5335	**-0.1864**	847.5339	**0.2855**	847.5363	**3.1173**
PI(36:5)	C45H76O13P	855.5024	855.5016	**-0.9351**	855.5031	**0.8182**	855.5058	**3.9743**
PI(36:4)	C45H78O13P	857.5180	_	_	_	_	_	_
PI(36:3)	C45H80O13P	859.5337	_	_	_	_	_	_
PI(36:2)	C45H82O13P	861.5493	_	_	_	_	_	_
PI-Cer identified as $[M-H]^-$								
PI-Cer(t18:0/16:0(2OH))	C40H79NO13P	812.5289	812.5260	**-3.5777**	812.5278	**-1.3624**	812.5294	**0.6067**
Cer identified as $[M+H]^+$								
Cer(d35:0)	C35H72NO3	554.5512	554.5511	**-0.2146**	554.5517	**0.8674**	554.5528	**2.8510**
MGMG identified as $[M+NH_4]^+$								
MGMG(16:3)	C25H46NO9	504.3173	504.3167	**-1.1084**	504.3171	**-0.3153**	504.3181	**1.6676**
MGMG(16:2)	C25H48NO9	506.3329	506.3335	**1.1672**	506.3339	**1.9572**	506.3349	**3.9322**
MGMG(16:0)	C25H52NO9	510.3642	_	_	_	_	_	_

MGMG(18:3)	C27H50NO9	532.3486	532.3485	**-0.1108**	532.3489	**0.6406**	532.3503	**3.2704**
MGMG(18:2)	C27H52NO9	534.3642	534.3638	**-0.7654**	534.3646	**0.7317**	534.3654	**2.2288**
MGDG identified as [M+NH_4]$^+$								
MGDG(32:6)	C41H70NO10	736.4994	736.4989	**-0.6789**	736.4998	**0.5431**	736.5012	**2.4440**
MGDG(32:5)	C41H72NO10	738.5156	738.5151	**-0.7095**	738.5153	**-0.4387**	738.5172	**2.1340**
MGDG(32:4)	C41H74NO10	740.5307	740.5284	**-3.1059**	740.5291	**-2.1606**	740.5306	**-0.1350**
MGDG(32:3)	C41H76NO10	742.5464	742.5446	**-2.4241**	742.5458	**-0.8080**	742.5468	**0.5387**
MGDG(32:2)	C41H78NO10	744.5626	744.5615	**-1.4774**	744.5630	**0.5372**	744.5665	**5.2380**
MGDG(34:7)	C43H72NO10	762.5156	_	_	_	_	_	_
MGDG(34:6)	C43H74NO10	764.5313	764.5311	**-0.2263**	764.5315	**0.2969**	764.5332	**2.5205**
MGDG(34:5)	C43H76NO10	766.5469	766.5446	**-3.0318**	766.5453	**-2.1186**	766.5471	**0.2296**
MGDG(34:4)	C43H78NO10	768.5626	768.5620	**-0.7807**	768.5624	**-0.2602**	768.5642	**2.0818**
MGDG(34:3)	C43H80NO10	770.5782	770.5783	**0.0986**	770.5743	**-5.0923**	770.5749	**-4.3136**
MGDG(34:2)	C43H82NO10	772.5933	772.5919	**-1.8121**	772.5927	**-0.7766**	772.5954	**2.7181**
MGDG(34:1)	C43H84NO10	774.6090	774.6058	**-4.1311**	774.6061	**-3.7438**	774.6065	**-3.2274**
MGDG(35:1)	C44H86NO10	788.6252	788.6242	**-1.2351**	788.6228	**-3.0103**	788.6249	**-0.3474**
MGDG(36:6)	C45H78NO10	792.5625	792.5635	**1.2617**	792.5643	**2.2711**	792.5656	**3.9114**
MGDG(36:5)	C45H80NO10	794.5782	794.5768	**-1.7619**	794.5773	**-1.1327**	794.5783	**0.1259**
MGDG(36:4)	C45H82NO10	796.5933	796.5918	**-1.8830**	796.5933	**0.0000**	796.5937	**0.5021**
MGDG(36:3)	C45H84NO10	798.6095	798.6062	**-4.1572**	798.6054	**-5.1590**	798.6091	**-0.5259**
MGDG(36:2)	C45H86NO10	800.6252	800.6212	**-4.9636**	800.6214	**-4.7138**	800.6240	**-1.4664**
DGMG identified as [M+NH_4]$^+$								
DGMG(16:0)	C31H62NO14	672.4170	672.4169	**-0.1487**	672.4185	**2.2308**	672.4188	**2.6769**
DGDG identified as [M+NH_4]$^+$								
DGDG(28:0)	C43H84O15N	854.5841	854.5852	**1.2872**	854.5864	**2.6914**	854.5886	**5.2657**
DGDG(32:5)	C47H82O15N	900.5684	900.5705	**2.3319**	900.5713	**3.2202**	900.5714	**3.3312**
DGDG(32:4)	C47H84O15N	902.5841	902.5845	**0.4432**	902.5849	**0.8863**	902.5856	**1.6619**
DGDG(32:3)	C47H86O15N	904.5997	904.6043	**5.0851**	904.6042	**4.9746**	904.6016	**2.1004**

DGDG(32:2)	C47H88O15N	906.6154	906.6173	**2.0957**	906.6179	**2.7575**	906.6200	**5.0738**
DGDG(32:1)	C47H90O15N	908.6310	908.6299	**-1.2106**	908.6295	**-1.6508**	908.6358	**5.2827**
DGDG(32:0)	C47H92O15N	910.6467	910.6476	**0.9883**	910.6497	**3.2944**	910.6505	**4.1729**
DGDG(34:6)	C49H84O15N	926.5841	926.5838	**-0.3238**	926.5848	**0.7555**	926.5871	**3.2377**
DGDG(34:5)	C49H86O15N	928.5997	928.5966	**-3.3384**	928.5976	**-2.2615**	928.6003	**0.6461**
DGDG(34:4)	C49H88O15N	930.6154	930.6145	**-0.9671**	930.6159	**0.5373**	930.6172	**1.9342**
DGDG(34:3)	C49H90O15N	932.6310	932.6276	**-3.6456**	932.6284	**-2.7878**	932.6303	**-0.7506**
DGDG(34:2)	C49H92O15N	934.6467	934.6461	**-0.6420**	934.6473	**0.6420**	934.6489	**2.3538**
DGDG(34:1)	C49H94O15N	936.6623	936.6577	**-4.9111**	936.6585	**-4.0570**	936.6611	**-1.2811**
DGDG(35:1)	C50H96NO15	950.6780	950.6769	**-1.1560**	950.6778	**-0.2093**	950.6805	**2.6308**
DGDG(35:2)	C50H94NO15	948.6623	948.6619	**-0.4733**	948.6629	**0.5808**	948.6648	**2.5836**
DGDG(35:3)	C50H92NO15	946.6467	946.6483	**1.6912**	946.6485	**1.9025**	946.6505	**4.0152**
DGDG(36:7)	C51H86O15N	952.5997	952.5967	**-3.1493**	952.5985	**-1.2597**	952.6019	**2.3095**
DGDG(36:6)	C51H88O15N	954.6154	954.6173	**1.9903**	954.6181	**2.8284**	954.6200	**4.8187**
DGDG(36:5)	C51H90O15N	956.6310	956.6318	**0.8363**	956.6327	**1.7771**	956.6340	**3.1360**
DGDG(36:4)	C51H92O15N	958.6467	958.6460	**-0.7302**	958.6460	**-0.7302**	958.6478	**1.1475**
DGDG(36:3)	C51H94O15N	960.6623	960.6582	**-4.2679**	960.6580	**-4.4761**	960.6638	**1.5614**
DGDG(36:2)	C51H96O15N	962.6780	_	_	_	_	_	_
SQDG identified as [M-H]$^-$								
SQDG(30:0)	C39H73O12S	765.4823	_	_	_	_	_	_
SQDG(32:3)	C41H71O12S	787.4666	787.4669	**0.3467**	787.4679	**1.6166**	787.4700	**4.2834**
SQDG(32:2)	C41H73O12S	789.4823	789.4828	**0.6625**	789.4839	**2.0558**	789.4862	**4.9691**
SQDG(32:1)	C41H75O12S	791.4979	791.4967	**-1.5502**	791.4980	**0.0922**	791.5007	**3.5035**
SQDG(32:0)	C41H77O12S	793.5136	793.5131	**-0.5999**	793.5141	**0.6604**	793.5164	**3.5589**
SQDG(34:5)	C43H71O12S	811.4666	811.4674	**0.9526**	811.4678	**1.4455**	811.4698	**3.9102**
SQDG(34:4)	C43H73O12S	813.4823	813.4823	**0.0283**	813.4826	**0.3971**	813.4851	**3.4703**
SQDG(34:3)	C43H75O12S	815.4979	815.4975	**-0.5236**	815.4985	**0.7026**	815.5008	**3.5230**
SQDG(34:2)	C43H77O12S	817.5136	817.5115	**-2.5394**	817.5124	**-1.4385**	817.5148	**1.4972**

SQDG(34:1)	C43H79O12S	819.5292	819.5288	**-0.5210**	819.5295	**0.3331**	819.5319	**3.2616**
SQDG(34:0)	C43H81O12S	821.5449	821.5439	**-1.1892**	821.5443	**-0.7023**	821.5473	**2.9493**
SQDG(36:6)	C45H73O12S	837.4823	837.4817	**-0.6890**	837.4829	**0.7439**	837.4850	**3.2514**
SQDG(36:5)	C45H75O12S	839.4979	839.4962	**-2.0572**	839.4966	**-1.5807**	839.4988	**1.0399**
SQDG(36:4)	C45H77O12S	841.5136	841.5142	**0.7415**	841.5150	**1.6922**	841.5173	**4.4254**
SQDG(36:3)	C45H79O12S	843.5292	843.5282	**-1.2175**	843.5307	**1.7462**	843.5318	**3.0503**
TG identified as $[M+NH_4]^+$								
TG(48:5)	C51H92NO6	814.6925	814.6906	**-2.2880**	814.6904	**-2.5335**	814.6924	**-0.0786**
TG(48:4)	C51H94NO6	816.7081	816.7095	**1.6971**	816.7101	**2.4317**	816.7116	**4.2684**
TG(48:3)	C51H96NO6	818.7238	818.7249	**1.3875**	818.7253	**1.8761**	818.7264	**3.2196**
TG(48:2)	C51H98NO6	820.7394	820.7367	**-3.3068**	820.7399	**0.5921**	820.7406	**1.4450**
TG(49:3)	C52H98NO6	832.7394	832.7368	**-3.1390**	832.7387	**-0.8574**	832.7414	**2.3849**
TG(49:2)	C52H100NO6	834.7551	834.7545	**-0.6756**	834.7570	**2.3192**	834.7567	**1.9599**
TG(50:7)	C53H92NO6	838.6925	838.6905	**-2.3417**	838.6918	**-0.7917**	838.6927	**0.2814**
TG(50:6)	C53H94NO6	840.7081	840.7077	**-0.4924**	840.7085	**0.4591**	840.7117	**4.2655**
TG(50:5)	C53H96NO6	842.7238	842.7234	**-0.4319**	842.7243	**0.6360**	842.7266	**3.3653**
TG(50:4)	C53H98NO6	844.7394	844.7373	**-2.5025**	844.7387	**-0.8452**	844.7407	**1.5224**
TG(50:3)	C53H100NO6	846.7551	846.7555	**0.5149**	846.7561	**1.2235**	846.7582	**3.7036**
TG(50:2)	C53H102NO6	848.7718	848.7705	**-1.5316**	848.7716	**-0.2356**	848.7736	**2.1207**
TG(50:1)	C53H104NO6	850.7864	850.7826	**-4.4241**	850.7845	**-2.1909**	850.7876	**1.4528**
TG(51:5)	C54H98NO6	856.7394	856.7394	**-0.0163**	856.7400	**0.6840**	856.7427	**3.8355**
TG(51:4)	C54H100NO6	858.7551	858.7558	**0.8571**	858.7562	**1.3228**	858.7585	**4.0011**
TG(51:3)	C54H102NO6	860.7707	860.7685	**-2.5721**	860.7702	**-0.5971**	860.7725	**2.0749**
TG(51:2)	C54H104NO6	862.7864	862.7844	**-2.2763**	862.7855	**-1.0014**	862.7872	**0.9690**
TG(52:8)	C55H94NO6	864.7081	864.7087	**0.6777**	864.7098	**1.9498**	864.7122	**4.7253**
TG(51:1)	C54H106NO6	864.8020	864.8009	**-1.2882**	864.8019	**-0.1318**	864.8050	**3.4528**
TG(52:7)	C55H96NO6	866.7238	866.7227	**-1.2276**	866.7245	**0.8492**	866.7246	**0.9646**
TG(52:6)	C55H98NO6	868.7394	868.7395	**0.0990**	868.7399	**0.5594**	868.7433	**4.4731**

TG(52:5)	$C_{55}H_{100}NO_6$	870.7551	870.7539	**-1.3368**	870.7553	**0.2710**	870.7579	**3.2569**
TG(52:4)	$C_{55}H_{102}NO_6$	872.7707	872.7686	**-2.4222**	872.7688	**-2.1930**	872.7705	**-0.2452**
TG(52:3)	$C_{55}H_{104}NO_6$	874.7864	874.7835	**-3.2739**	874.7840	**-2.7024**	874.7864	**0.0412**
TG(52:2)	$C_{55}H_{106}NO_6$	876.8020	876.7978	**-4.8061**	876.8005	**-1.7267**	876.8018	**-0.2441**
TG(52:1)	$C_{55}H_{108}NO_6$	878.8177	878.8180	**0.3823**	878.8171	**-0.6418**	878.8204	**3.1133**
TG(53:5)	$C_{56}H_{102}NO_6$	884.7707	884.7722	**1.6795**	884.7731	**2.6967**	884.7750	**4.8442**
TG(53:4)	$C_{56}H_{104}NO_6$	886.7864	886.7844	**-2.2147**	886.7861	**-0.2977**	886.7879	**1.7321**
TG(53:3)	$C_{56}H_{106}NO_6$	888.8020	888.8023	**0.3218**	888.8038	**2.0094**	888.8060	**4.4847**
TG(53:2)	$C_{56}H_{108}NO_6$	890.8177	890.8175	**-0.1841**	890.8171	**-0.6331**	890.8207	**3.4081**
TG(54:7)	$C_{57}H_{100}NO_6$	894.7551	894.7531	**-2.1950**	894.7534	**-1.8597**	894.7555	**0.4873**
TG(54:6)	$C_{57}H_{102}NO_6$	896.7707	896.7707	**-0.0156**	896.7725	**1.9916**	896.7709	**0.2074**
TG(54:5)	$C_{57}H_{104}NO_6$	898.7864	898.7841	**-2.5190**	898.7851	**-1.4063**	898.7890	**2.9328**
TG(54:4)	$C_{57}H_{106}NO_6$	900.8020	900.7976	**-4.9001**	900.7998	**-2.4578**	900.8010	**-1.1257**
TG(54:3)	$C_{57}H_{108}NO_6$	902.8177	902.8184	**0.8152**	902.8166	**-1.1785**	902.8195	**2.0336**
TG(54:2)	$C_{57}H_{110}NO_6$	904.8333	904.8315	**-2.0048**	904.8329	**-0.4575**	904.8339	**0.6476**
Pigments identified as [M+H]$^+$								
Chlorophyll b	$C_{55}H_{71}MgN_4O_6$	907.5224	907.5220	**-0.4474**	907.5237	**1.4259**	907.5250	**2.8583**
Neoxanthin or Violaxanthin	$C_{40}H_{57}O_4$	601.4257	601.4249	**-1.3052**	601.4249	**-1.3052**	601.4261	**0.6900**
Antheraxanthin	$C_{40}H_{57}O_3$	585.4308	585.4309	**0.2238**	585.4295	**-2.1676**	585.4315	**1.2487**
Astaxanthin	$C_{40}H_{53}O_4$	597.3944	597.3937	**-1.1466**	597.3943	**-0.1423**	597.3955	**1.8664**
Pheophorbide a	$C_{35}H_{37}N_4O_5$	593.2764	593.2762	**-0.3304**	593.2767	**0.5124**	593.2780	**2.7036**
Pheophytin b	$C_{55}H_{73}N_4O_6$	885.5530	885.5522	**-0.9158**	885.5532	**0.2134**	885.5553	**2.5848**
Pheophorbide b	$C_{35}H_{35}N_4O_6$	607.2557	607.2552	**-0.7592**	607.2559	**0.3936**	607.2573	**2.6990**
Pheophytin a	$C_{55}H_{75}N_4O_5$	871.5737	871.5737	**-0.0528**	871.5747	**1.0946**	871.5765	**3.1598**
Chlorophyll-a	$C_{55}H_{73}MgN_4O_5$	893.5431	893.5436	**0.5137**	893.5458	**2.9758**	893.5473	**4.6545**

Abbreviations: C-Auto, *C. vulgaris* cultivated under autotrophic conditions; DCM, dichloromethane:methanol (2:1, v/v); EtOH, ethanol, UAE, ethanol extraction assisted by an ultrasound probe; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; Cer, ceramide; PI_Cer, inositolphosphoceramide; TG, triglyceride.

Table S3.2. Exact mass measurements of the molecular ions identified by HILIC−ESI−MS in the DCM, EtOH, and UAE extracts from *Chlorella vulgaris* cultivated under heterotrophic conditions (C-Hetero). C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains.

Lipid species (C:N)	Formula	Calculated *m/z*	Observed *m/z* in MS of the DCM extracts	Error (ppm)	Observed *m/z* in MS of the UAE extracts	Error (ppm)	Observed *m/z* in MS of the Ethanol extracts	Error (ppm)
PC identified as $[M+H]^+$								
PC(30:3)	C38H71NO8P	700.4917	700.4925	**1.0964**	700.4931	**1.9529**	700.4926	**1.2391**
PC(32:6)	C40H69NO8P	722.4761	722.4797	**5.0078**	722.4797	**5.0078**	722.4797	**5.0078**
PC(32:5)	C40H71NO8P	724.4917	724.4952	**4.7868**	724.4951	**4.6488**	724.4952	**4.7868**
PC(32:4)	C40H73NO8P	726.5074	726.5106	**4.4294**	726.5105	**4.2918**	726.5107	**4.5671**
PC(32:3)	C40H75NO8P	728.5230	728.5260	**4.0740**	728.5263	**4.4858**	728.5262	**4.3485**
PC(32:2)	C40H77NO8P	730.5387	730.5418	**4.2681**	730.5416	**3.9943**	730.5414	**3.7205**
PC(32:1)	C40H79NO8P	732.5543	732.5541	**-0.3153**	732.5542	**-0.1788**	732.5539	**-0.5884**
PC(32:0)	C40H81NO8P	734.5700	734.5721	**2.8833**	734.5714	**1.9304**	734.5722	**3.0195**
PC(33:2)	C41H79NO8P	744.5543	744.5575	**4.2562**	744.5573	**3.9876**	744.5576	**4.3905**
PC(33:3)	C41H77NO8P	742.5387	742.5418	**4.1991**	742.5417	**4.0644**	742.5416	**3.9298**
PC(34:8)	C42H69NO8P	746.4761	746.4775	**1.8996**	746.4776	**2.0336**	746.4777	**2.1675**
PC(34:7)	C42H71NO8P	748.4917	748.4921	**0.4917**	748.4921	**0.4917**	748.4919	**0.2245**
PC(34:6)	C42H73NO8P	750.5074	750.5094	**2.6888**	750.5096	**2.9553**	750.5095	**2.8221**
PC(34:5(OH))	C42H75NO9P	768.5179	_	_	_	_	_	_
PC(34:5)	C42H75NO8P	752.5230	752.5257	**3.5454**	752.5255	**3.2796**	752.5258	**3.6783**

PC(34:4)	C42H77NO8P	754.5387	754.5416	**3.8673**	754.5415	**3.7347**	754.5418	**4.1323**
PC(34:3)	C42H79NO8P	756.5543	756.5570	**3.5278**	756.5574	**4.0565**	756.5571	**3.6600**
PC(34:2)	C42H81NO8P	758.5700	758.5728	**3.7149**	758.5729	**3.8467**	758.5731	**4.1104**
PC(34:1)	C42H83NO8P	760.5856	760.5872	**2.0616**	760.5870	**1.7986**	760.5873	**2.1930**
PC(35:1)	C43H85NO8P	774.6013	774.6033	**2.6052**	774.6031	**2.3470**	774.6032	**2.4761**
PC(35:2)	C43H83NO8P	772.5856	772.5887	**3.9711**	772.5884	**3.5828**	772.5886	**3.8416**
PC(35:3)	C43H81NO8P	770.5700	770.5726	**3.3975**	770.5726	**3.3975**	770.5728	**3.6570**
PC(35:4)	C43H79NO8P	768.5543	768.5566	**2.9523**	768.5564	**2.6921**	768.5566	**2.9523**
PC(36:9)	C44H71NO8P	772.4917	772.4921	**0.4764**	772.4942	**3.1949**	772.4935	**2.2887**
PC(36:8)	C44H73NO8P	774.5074	774.5082	**1.0562**	774.5087	**1.7017**	774.5085	**1.4435**
PC(36:7)	C44H75NO8P	776.5230	776.5228	**-0.2988**	776.5233	**0.3451**	776.5233	**0.3451**
PC(36:6)	C44H77NO8P	778.5387	778.5402	**1.9498**	778.5399	**1.5645**	778.5401	**1.8214**
PC(36:5)	C44H79NO8P	780.5543	780.5566	**2.9069**	780.5564	**2.6507**	780.5564	**2.6507**
PC(36:4(OH))	C44H81NO9P	798.5649	798.5678	**3.6353**	798.5673	**3.0091**	798.5676	**3.3848**
PC(36:4)	C44H81NO8P	782.5700	782.5729	**3.7287**	782.5726	**3.3454**	782.5730	**3.8565**
PC(36:3)	C44H83NO8P	784.5856	784.5874	**2.2534**	784.5876	**2.5083**	784.5873	**2.1260**
PC(36:2)	C44H85NO8P	786.6013	786.6019	**0.7857**	786.6021	**1.0399**	786.6020	**0.9128**
PC(36:1)	C44H87NO8P	788.6169	788.6149	**-2.5767**	788.6148	**-2.7035**	788.6153	**-2.0694**
PC(37:2)	C45H87NO8P	800.6169	800.6185	**1.9585**	800.6183	**1.7087**	800.6180	**1.3340**
PC(37:3)	C45H85NO8P	798.6013	798.6042	**3.6539**	798.6040	**3.4035**	798.6042	**3.6539**
PC(37:4)	C45H83NO8P	796.5856	796.5865	**1.0897**	796.5865	**1.0897**	796.5864	**0.9641**
PC(37:5)	C45H81NO8P	794.5700	794.5700	**0.0227**	794.5698	**-0.2291**	794.5702	**0.2744**
PC(38:9)	C46H75NO8P	800.5230	800.5241	**1.3341**	800.5211	**-2.4134**	800.5195	**-4.4121**
PC(38:8)	C46H77NO8P	802.5387	802.5387	**0.0224**	802.5393	**0.7701**	802.5390	**0.3962**
PC(38:7)	C46H79NO8P	804.5543	804.5550	**0.8315**	804.5547	**0.4586**	804.5548	**0.5829**

PC(38:6)	C46H81NO8P	806.5700	806.5696	**-0.4736**	806.5698	**-0.2256**	806.5694	**-0.7216**
PC(38:5)	C46H83NO8P	808.5856	808.5844	**-1.5236**	808.5842	**-1.7710**	808.5837	**-2.3894**
PC(38:4)	C46H85NO8P	810.6013	810.6021	**1.0091**	810.6028	**1.8727**	810.6018	**0.6390**
PC(38:3)	C46H87NO8P	812.6169	812.6189	**2.4218**	812.6188	**2.2987**	812.6191	**2.6679**
PC(38:2)	C46H89NO8P	814.6326	814.6331	**0.6359**	814.6344	**2.2317**	814.6342	**1.9862**
LPC identified as [M+H]$^+$								
LPC(16:3)	C24H45NO7P	490.2934	_	_	_	_	_	_
LPC(16:2)	C24H47NO7P	492.3090	492.3099	**1.7936**	492.3092	**0.3717**	492.3096	**1.1842**
LPC(16:1)	C24H49NO7P	494.3247	_	_	_	_	_	_
LPC(16:0)	C24H51NO7P	496.3403	496.3422	**3.7958**	496.3423	**3.9973**	496.3422	**3.7958**
LPC(18:3)	C26H49NO7P	518.3247	518.3260	**2.5717**	518.3264	**3.3435**	518.3261	**2.7647**
LPC(18:2)	C26H51NO7P	520.3403	520.3425	**4.1973**	520.3425	**4.1973**	520.3426	**4.3894**
LPC(18:1)	C26H53NO7P	522.3560	522.3583	**4.4663**	522.3582	**4.2749**	522.3583	**4.4663**
LPC(18:0)	C26H55NO7P	524.3716	_	_	_	_	_	_
LPC(20:5)	C28H49NO7P	542.3247	_	_	_	_	_	_
LPC(20:4)	C28H51NO7P	544.3403	_	_	_	_	_	_
PE identified as [M+H]$^+$								
PE(30:3)	C35H65NO8P	658.4448	658.4452	**0.6348**	658.4456	**1.2423**	658.4452	**0.6348**
PE(30:2)	C35H67NO8P	660.4604	660.4594	**-1.5625**	660.4637	**4.9481**	660.4597	**-1.1083**
PE(30:1)	C35H69NO8P	662.4761	_	_	_	_	_	_
PE(30:0)	C35H71NO8P	664.4917	_	_	_	_	_	_
PE(32:6)	C37H63NO8P	680.4291	680.4269	**-3.2803**	680.4262	**-4.3090**	680.4257	**-5.0439**
PE(32:5)	C37H65NO8P	682.4448	682.4478	**4.4223**	682.4480	**4.7154**	682.4482	**5.0085**
PE(32:4)	C37H67NO8P	684.4604	684.4638	**4.9207**	684.4637	**4.7746**	684.4638	**4.9207**
PE(32:3)	C37H69NO8P	686.4761	686.4789	**4.1050**	686.4791	**4.3964**	686.4789	**4.1050**

PE(32:2)	C37H71O8NP	688.4917	688.4947	**4.3109**	688.4951	**4.8919**	688.4949	**4.6014**
PE(32:1)	C37H73NO8P	690.5074	690.5079	**0.7502**	690.5079	**0.7502**	690.5079	**0.7502**
PE(33:3)	C38H71NO8P	700.4917	700.4950	**4.6653**	700.4949	**4.5225**	700.4952	**4.9508**
PE(34:6)	C39H67NO8P	708.4604	708.4624	**2.7779**	708.4622	**2.4956**	708.4625	**2.9190**
PE(34:5)	C39H69NO8P	710.4761	710.4782	**2.9811**	710.4781	**2.8403**	710.4782	**2.9811**
PE(34:4)	C39H71NO8P	712.4917	712.4950	**4.5867**	712.4949	**4.4464**	712.4951	**4.7271**
PE(34:3)	C39H73O8NP	714.5074	714.5107	**4.6438**	714.5108	**4.7837**	714.5107	**4.6438**
PE(34:2)	C39H75NO8P	716.5230	716.5260	**4.1422**	716.5264	**4.7005**	716.5262	**4.4214**
PE(34:1)	C39H77NO8P	718.5387	718.5399	**1.6951**	718.5402	**2.1126**	718.5399	**1.6951**
PE(35:2)	C40H77NO8P	730.5387	730.5416	**3.9943**	730.5416	**3.9943**	730.5416	**3.9943**
PE(35:3)	C40H75NO8P	728.5230	728.5257	**3.6622**	728.5260	**4.0740**	728.5259	**3.9367**
PE(35:4)	C40H73NO8P	726.5074	726.5093	**2.6400**	726.5090	**2.2271**	726.5090	**2.2271**
PE(36:9)	C41H65NO8P	730.4448	730.4446	**-0.2492**	730.4450	**0.2984**	730.4451	**0.4354**
PE(36:8)	C41H67NO8P	732.4604	732.4602	**-0.3167**	732.4602	**-0.3167**	732.4602	**-0.3167**
PE(36:7)	C41H69NO8P	734.4761	734.4771	**1.3860**	734.4767	**0.8414**	734.4767	**0.8414**
PE(36:6)	C41H71NO8P	736.4917	736.4934	**2.2648**	736.4936	**2.5363**	736.4934	**2.2648**
PE(36:5)	C41H73O8NP	738.5074	738.5095	**2.8679**	738.5094	**2.7325**	738.5095	**2.8679**
PE(36:4(OH))	C41H75NO9P	756.5179	756.5204	**3.2425**	756.5195	**2.0528**	756.5202	**2.9781**
PE(36:4)	C41H75NO8P	740.5230	740.5261	**4.1430**	740.5259	**3.8729**	740.5261	**4.1430**
PE(36:3)	C41H77NO8P	742.5387	742.5391	**0.5629**	742.5395	**1.1016**	742.5391	**0.5629**
PE(36:2)	C41H79O8NP	744.5543	744.5569	**3.4504**	744.5571	**3.7190**	744.5571	**3.7190**
PE(37:5)	C42H75NO8P	752.5230	752.5238	**1.0206**	752.5238	**1.0206**	752.5240	**1.2863**
PE(38:9)	C43H69NO8P	758.4761	758.4729	**-4.1953**	758.4738	**-3.0087**	758.4737	**-3.1405**
PE(38:8)	C43H71NO8P	760.4917	760.4915	**-0.3051**	760.4912	**-0.6995**	760.4912	**-0.6995**
PE(38:7)	C43H73NO8P	762.5074	762.5077	**0.4170**	762.5082	**1.0728**	762.5078	**0.5482**

LPE identified as [M+H]$^+$								
LPE(16:1)	C21H43NO7P	452.2777	_	_	_	_	_	_
LPE(16:0)	C21H45NO7P	454.2934	454.2951	**3.8147**	454.2954	**4.4751**	454.2952	**4.0348**
LPE(18:3)	C23H43NO7P	476.2777	_	_	_	_	_	_
LPE(18:2)	C23H45NO7P	478.2934	478.2953	**4.0415**	478.2955	**4.4596**	478.2953	**4.0415**
LPE(18:1)	C23H47NO7P	480.3090	480.3113	**4.7532**	480.3109	**3.9204**	480.3108	**3.7122**
LPE(20:5)	C25H43NO7P	500.2777	500.2770	**-1.4332**	500.2767	**-2.0329**	500.2773	**-0.8335**
LPE(20:4)	C25H45NO7P	502.2934	_	_	_	_	_	_
PG identified as [M-H]$^-$								
PG(32:1)	C38H72O10P	719.4863	719.4879	**2.2238**	719.4885	**3.0577**	719.4857	**-0.8339**
PG(32:0)	C38H74O10P	721.5020	721.5040	**2.7720**	721.5050	**4.1580**	721.5018	**-0.2772**
PG(33:0)	C39H76O10P	735.5176	735.5196	**2.7015**	735.5205	**3.9251**	735.5176	**-0.0177**
PG(34:4)	C40H70O10P	741.4707	741.4699	**-1.0789**	741.4703	**-0.5395**	741.4672	**-4.7203**
PG(34:3)	C40H72O10P	743.4863	743.4877	**1.8830**	743.4888	**3.3625**	743.4855	**-1.0760**
PG(34:2)	C40H74O10P	745.5020	745.5039	**2.5486**	745.5048	**3.7559**	745.5017	**-0.4024**
PG(34:1)	C40H76O10P	747.5176	747.5192	**2.1404**	747.5203	**3.6120**	747.5169	**-0.9364**
PG(34:0)	C40H78O10P	749.5333	749.5305	**-3.7357**	749.5301	**-4.2693**	749.5304	**-3.8691**
PG(36:5)	C42H72O10P	767.4863	767.4845	**-2.3453**	767.4863	**0.0000**	767.4832	**-4.0392**
PG(36:4)	C42H74O10P	769.5020	769.5025	**0.6498**	769.5043	**2.9889**	769.5009	**-1.4295**
PG(36:3)	C42H76O10P	771.5176	771.5188	**1.5554**	771.5194	**2.3331**	771.5159	**-2.2034**
PG(36:2)	C42H78O10P	773.5333	773.5333	**0.0000**	773.5344	**1.4220**	773.5317	**-2.0684**
PG(36:1)	C42H80O10P	775.5489	775.5500	**1.4184**	775.5510	**2.7078**	775.5480	**-1.1605**
PG(36:0)	C42H82O10P	777.5646	777.5643	**-0.3858**	777.5645	**-0.1286**	777.5616	**-3.8582**
PG(38:5)	C44H76O10P	795.5176	795.5199	**2.8912**	795.5214	**4.7768**	795.5179	**0.3771**
PI identified as [M-H]$^-$								

PI(32:2)	C41H74O13P	805.4867	805.4850	**-2.1105**	805.4841	**-3.2279**	805.4844	**-2.8554**
PI(34:3)	C43H76O13P	831.5024	831.5041	**2.0445**	831.5051	**3.2471**	831.5014	**-1.2026**
PI(34:2)	C43H78O13P	833.5180	833.5202	**2.6394**	833.5214	**4.0791**	833.5177	**-0.3599**
PI(34:1)	C43H80O13P	835.5337	835.5343	**0.7181**	835.5352	**1.7953**	835.5321	**-1.9149**
PI(35:2)	C44H80O13P	847.5337	847.5352	**1.8194**	847.5364	**3.2353**	847.5329	**-0.8944**
PI(36:5)	C45H76O13P	855.5024	855.5043	**2.2209**	855.5052	**3.2729**	855.5019	**-0.5845**
PI(36:4)	C45H78O13P	857.5180	857.5198	**2.0991**	857.5205	**2.9154**	857.5174	**-0.6997**
PI(36:3)	C45H80O13P	859.5337	859.5350	**1.5124**	859.5360	**2.6759**	859.5327	**-1.1634**
PI(36:2)	C45H82O13P	861.5493	861.5499	**0.6964**	861.5510	**1.9732**	861.5477	**-1.8571**
PI_Cer identified as $[M-H]^-$								
PI-Cer(t18:0/16:0(2OH))	C40H79NO13P	812.5289	812.5259	**-3.7008**	812.5255	**-4.1931**	812.5256	**-4.0700**
Cer identified as $[M+H]^+$								
Cer(d35:0)	C35H72NO3	554.5512	554.5529	**3.0313**	554.5535	**4.1132**	554.5540	**5.0149**
MGMG identified as $[M+NH_4]^+$								
MGMG(16:3)	C25H46NO9	504.3173	504.3189	**3.2539**	504.3192	**3.8488**	504.3192	**3.8488**
MGMG(16:2)	C25H48NO9	506.3329	506.3352	**4.5247**	506.3351	**4.3272**	506.3352	**4.5247**
MGMG(16:0)	C25H52NO9	510.3642	510.3643	**0.1959**	510.3664	**4.3106**	510.3661	**3.7228**
MGMG(18:3)	C27H50NO9	532.3486	_	_	_	_	_	_
MGMG(18:2)	C27H52NO9	534.3642	534.3657	**2.7902**	534.3663	**3.9131**	534.3664	**4.1002**
MGDG identified as $[M+NH_4]^+$								
MGDG(32:6)	C41H70NO10	736.4994	_	_	_	_	_	_
MGDG(32:5)	C41H72NO10	738.5156	738.5142	**-1.9282**	738.5147	**-1.2512**	738.5132	**-3.2823**
MGDG(32:4)	C41H74NO10	740.5307	740.5317	**1.3504**	740.5337	**4.0511**	740.5320	**1.7555**
MGDG(32:3)	C41H76NO10	742.5464	742.5470	**0.8080**	742.5470	**0.8080**	742.5464	**0.0000**
MGDG(32:2)	C41H78NO10	744.5626	744.5636	**1.3431**	744.5641	**2.0146**	744.5656	**4.0292**

MGDG(34:7)	C43H72NO10	762.5156	762.5141	**-1.9986**	762.5186	**3.9029**	762.5184	**3.6406**
MGDG(34:6)	C43H74NO10	764.5313	764.5336	**3.0437**	764.5341	**3.6977**	764.5338	**3.3053**
MGDG(34:5)	C43H76NO10	766.5469	766.5494	**3.2301**	766.5495	**3.3605**	766.5492	**2.9692**
MGDG(34:4)	C43H78NO10	768.5626	768.5652	**3.3829**	768.5655	**3.7733**	768.5653	**3.5131**
MGDG(34:3)	C43H80NO10	770.5782	770.5802	**2.5643**	770.5802	**2.5643**	770.5799	**2.1750**
MGDG(34:2)	C43H82NO10	772.5933	772.5968	**4.5302**	772.5972	**5.0479**	772.5969	**4.6596**
MGDG(34:1)	C43H84NO10	774.6090	774.6124	**4.3893**	774.6123	**4.2602**	774.6124	**4.3893**
MGDG(34:0)	C43H86NO10	776.6246	776.6213	**-4.2492**	776.6208	**-4.8930**	776.6208	**-4.8930**
MGDG(35:1)	C44H86NO10	788.6252	788.6288	**4.5979**	788.6289	**4.7247**	788.6251	**-0.0938**
MGDG(36:6)	C45H78NO10	792.5625	792.5657	**4.0375**	792.5653	**3.5328**	792.5648	**2.9020**
MGDG(36:5)	C45H80NO10	794.5782	794.5810	**3.5239**	794.5816	**4.2790**	794.5813	**3.9014**
MGDG(36:4)	C45H82NO10	796.5933	796.5964	**3.8916**	796.5966	**4.1426**	796.5961	**3.5150**
MGDG(36:3)	C45H84NO10	798.6095	798.6069	**-3.2807**	798.6084	**-1.4024**	798.6074	**-2.6546**
MGDG(36:2)	C45H86NO10	800.6252	800.6223	**-3.5897**	800.6240	**-1.4664**	800.6229	**-2.8403**
DGMG identified as $[M+NH_4]^+$								
DGMG(16:0)	C31H62NO14	672.4170	672.4197	**4.0154**	672.4196	**3.8666**	672.4199	**4.3128**
DGDG identified as $[M+NH_4]^+$								
DGDG(28:0)	C43H84O15N	854.5841	854.5862	**2.4573**	854.5799	**-4.9147**	854.5854	**1.5212**
DGDG(32:5)	C47H82O15N	900.5684	_	_	_	_	_	_
DGDG(32:4)	C47H84O15N	902.5841	_	_	_	_	_	_
DGDG(32:3)	C47H86O15N	904.5997	904.6039	**4.6429**	904.6035	**4.2008**	904.6026	**3.2058**
DGDG(32:2)	C47H88O15N	906.6154	906.6200	**5.0738**	906.6195	**4.5223**	906.6199	**4.9635**
DGDG(32:1)	C47H90O15N	908.6310	908.6354	**4.8424**	908.6353	**4.7324**	908.6339	**3.1916**
DGDG(32:0)	C47H92O15N	910.6467	910.6510	**4.7219**	910.6511	**4.8317**	910.6505	**4.1729**
DGDG(34:6)	C49H84O15N	926.5841	926.5883	**4.5328**	926.5884	**4.6407**	926.5879	**4.1011**

DGDG(34:5)	C49H86O15N	928.5997	928.6032	**3.7691**	928.6031	**3.6614**	928.6040	**4.6306**
DGDG(34:4)	C49H88O15N	930.6154	930.6198	**4.7281**	930.6194	**4.2982**	930.6198	**4.7281**
DGDG(34:3)	C49H90O15N	932.6310	932.6335	**2.6806**	932.6337	**2.8950**	932.6331	**2.2517**
DGDG(34:2)	C49H92O15N	934.6467	934.6510	**4.6007**	934.6507	**4.2797**	934.6512	**4.8147**
DGDG(34:1)	C49H94O15N	936.6623	936.6655	**3.4164**	936.6654	**3.3096**	936.6653	**3.2029**
DGDG(35:1)	C50H96NO15	950.6780	950.6820	**4.2086**	950.6818	**3.9982**	950.6818	**3.9982**
DGDG(35:2)	C50H94NO15	948.6623	948.6670	**4.9027**	948.6669	**4.7973**	948.6670	**4.9027**
DGDG(35:3)	C50H92NO15	946.6467	946.6483	**1.6912**	946.6505	**4.0152**	946.6503	**3.8040**
DGDG(36:7)	C51H86O15N	952.5997	952.6030	**3.4642**	952.6037	**4.1990**	952.6032	**3.6742**
DGDG(36:6)	C51H88O15N	954.6154	954.6199	**4.7139**	954.6189	**3.6664**	954.6182	**2.9331**
DGDG(36:5)	C51H90O15N	956.6310	956.6353	**4.4949**	956.6353	**4.4949**	956.6357	**4.9131**
DGDG(36:4)	C51H92O15N	958.6467	958.6503	**3.7553**	958.6509	**4.3812**	958.6504	**3.8596**
DGDG(36:3)	C51H94O15N	960.6623	960.6633	**1.0409**	960.6628	**0.5205**	960.6641	**1.8737**
DGDG(36:2)	C51H96O15N	962.6780	962.6788	**0.8310**	962.6776	**-0.4155**	962.6778	**-0.2078**
SQDG identified as [M-H]$^-$								
SQDG(30:0)	C39H73O12S	765.4823	765.4838	**1.9896**	765.4850	**3.5572**	765.4822	**-0.1006**
SQDG(32:3)	C41H71O12S	787.4666	787.4685	**2.3785**	787.4697	**3.9024**	787.4664	**-0.2883**
SQDG(32:2)	C41H73O12S	789.4823	789.4842	**2.4358**	789.4852	**3.7024**	789.4822	**-0.0975**
SQDG(32:1)	C41H75O12S	791.4979	791.5003	**2.9981**	791.5017	**4.7669**	791.4992	**1.6083**
SQDG(32:0)	C41H77O12S	793.5136	793.5157	**2.6767**	793.5165	**3.6849**	793.5136	**0.0302**
SQDG(34:5)	C43H71O12S	811.4666	811.4691	**3.0476**	811.4706	**4.8961**	811.4673	**0.8294**
SQDG(34:4)	C43H73O12S	813.4823	813.4843	**2.4868**	813.4853	**3.7161**	813.4822	**-0.0947**
SQDG(34:3)	C43H75O12S	815.4979	815.5000	**2.5420**	815.5009	**3.6456**	815.4980	**0.0895**
SQDG(34:2)	C43H77O12S	817.5136	817.5153	**2.1088**	817.5165	**3.5767**	817.5131	**-0.5823**
SQDG(34:1)	C43H79O12S	819.5292	819.5306	**1.6754**	819.5318	**3.1396**	819.5284	**-1.0091**

SQDG(34:0)	C43H81O12S	821.5449	821.5416	**-3.9888**	821.5419	**-3.6237**	821.5409	**-4.8409**
SQDG(36:6)	C45H73O12S	837.4823	_	_	_	_	_	_
SQDG(36:5)	C45H75O12S	839.4979	839.4998	**2.2311**	839.5000	**2.4693**	839.4977	**-0.2704**
SQDG(36:4)	C45H77O12S	841.5136	841.5156	**2.4052**	841.5162	**3.1182**	841.5140	**0.5039**
SQDG(36:3)	C45H79O12S	843.5292	843.5308	**1.8648**	843.5325	**3.8801**	843.5295	**0.3236**
TG identified as [M+NH$_4$]$^+$								
TG(48:5)	C51H92NO6	814.6925	814.6955	**3.7266**	814.6965	**4.9540**	814.6959	**4.2175**
TG(48:4)	C51H94NO6	816.7081	816.7121	**4.8806**	816.7105	**2.9215**	816.7122	**5.0030**
TG(48:3)	C51H96NO6	818.7238	818.7274	**4.4411**	818.7279	**5.0518**	818.7277	**4.8075**
TG(48:2)	C51H98NO6	820.7394	820.7419	**3.0290**	820.7415	**2.5416**	820.7420	**3.1508**
TG(49:3)	C52H98NO6	832.7394	832.7422	**3.3456**	832.7409	**1.7845**	832.7411	**2.0246**
TG(49:2)	C52H100NO6	834.7551	834.7586	**4.2360**	834.7589	**4.5954**	834.7591	**4.8350**
TG(50:7)	C53H92NO6	838.6925	838.6956	**3.7392**	838.6953	**3.3815**	838.6956	**3.7392**
TG(50:6)	C53H94NO6	840.7081	840.7119	**4.5033**	840.7123	**4.9791**	840.7120	**4.6223**
TG(50:5)	C53H96NO6	842.7238	842.7271	**3.9586**	842.7274	**4.3146**	842.7272	**4.0773**
TG(50:4)	C53H98NO6	844.7394	844.7425	**3.6532**	844.7423	**3.4164**	844.7427	**3.8900**
TG(50:3)	C53H100NO6	846.7551	846.7580	**3.4674**	846.7581	**3.5855**	846.7576	**2.9950**
TG(50:2)	C53H102NO6	848.7718	848.7742	**2.8276**	848.7744	**3.0632**	848.7743	**2.9454**
TG(50:1)	C53H104NO6	850.7864	850.7875	**1.3352**	850.7874	**1.2177**	850.7873	**1.1002**
TG(51:5)	C54H98NO6	856.7394	856.7431	**4.3024**	856.7437	**5.0027**	856.7437	**5.0027**
TG(51:4)	C54H100NO6	858.7551	858.7586	**4.1176**	858.7587	**4.2340**	858.7589	**4.4669**
TG(51:3)	C54H102NO6	860.7707	860.7736	**3.3528**	860.7738	**3.5852**	860.7736	**3.3528**
TG(51:2)	C54H104NO6	862.7864	862.7899	**4.0983**	862.7899	**4.0983**	862.7897	**3.8665**
TG(52:8)	C55H94NO6	864.7081	864.7120	**4.4940**	864.7119	**4.3784**	864.7117	**4.1471**
TG(51:1)	C54H106NO6	864.8020	864.8050	**3.4528**	864.8054	**3.9153**	864.8046	**2.9903**

TG(52:7)	C55H96NO6	866.7238	866.7275	**4.3105**	866.7277	**4.5412**	866.7274	**4.1951**
TG(52:6)	C55H98NO6	868.7394	868.7434	**4.5883**	868.7432	**4.3580**	868.7434	**4.5883**
TG(52:5)	C55H100NO6	870.7551	870.7589	**4.4054**	870.7589	**4.4054**	870.7584	**3.8312**
TG(52:4)	C55H102NO6	872.7707	872.7740	**3.7650**	872.7734	**3.0776**	872.7733	**2.9630**
TG(52:3)	C55H104NO6	874.7864	874.7891	**3.1276**	874.7888	**2.7847**	874.7888	**2.7847**
TG(52:2)	C55H106NO6	876.8020	876.8039	**2.1510**	876.8036	**1.8088**	876.8035	**1.6948**
TG(52:1)	C55H108NO6	878.8177	878.8168	**-0.9831**	878.8174	**-0.3004**	878.8178	**0.1548**
TG(53:5)	C56H102NO6	884.7707	884.7750	**4.8442**	884.7746	**4.3921**	884.7751	**4.9572**
TG(53:4)	C56H104NO6	886.7864	886.7897	**3.7619**	886.7896	**3.6491**	886.7901	**4.2130**
TG(53:3)	C56H106NO6	888.8020	888.8051	**3.4721**	888.8055	**3.9221**	888.8045	**2.7970**
TG(53:2)	C56H108NO6	890.8177	890.8219	**4.7552**	890.8217	**4.5307**	890.8220	**4.8674**
TG(54:7)	C57H100NO6	894.7551	894.7594	**4.8460**	894.7583	**3.6166**	894.7590	**4.3990**
TG(54:6)	C57H102NO6	896.7707	896.7750	**4.7794**	896.7752	**5.0024**	896.7748	**4.5563**
TG(54:5)	C57H104NO6	898.7864	898.7900	**4.0455**	898.7897	**3.7117**	898.7894	**3.3779**
TG(54:4)	C57H106NO6	900.8020	900.8042	**2.4267**	900.8044	**2.6488**	900.8045	**2.7598**
TG(54:3)	C57H108NO6	902.8177	902.8200	**2.5875**	902.8199	**2.4767**	902.8199	**2.4767**
TG(54:2)	C57H110NO6	904.8333	904.8341	**0.8687**	904.8349	**1.7528**	904.8347	**1.5318**
Pigments identified as [M+H]$^+$								
Chlorophyll b	C55H71MgN4O6	907.5224	907.5261	**4.0704**	907.5266	**4.6214**	907.5261	**4.0704**
Neoxanthin or Violaxanthin	C40H57O4	601.4257	601.4279	**3.6829**	601.4265	**1.3551**	601.4270	**2.1865**
Antheraxanthin	C40H57O3	585.4308	585.4299	**-1.4844**	585.4303	**-0.8011**	585.4295	**-2.1676**
Astaxanthin	C40H53O4	597.3944	597.3973	**4.8795**	597.3970	**4.3773**	597.3965	**3.5404**
Pheophorbide a	C35H37N4O5	593.2764	593.2791	**4.5577**	593.2788	**4.0521**	593.2788	**4.0521**
Pheophytin b	C55H73N4O6	885.553	885.5565	**3.9399**	885.5562	**3.6011**	885.5564	**3.8270**
Pheophorbide b	C35H35N4O6	607.2557	607.2582	**4.1811**	607.2581	**4.0164**	607.2582	**4.1811**

Pheophytin a	C55H75N4O5	871.5737	871.5780	**4.8808**	871.5777	**4.5366**	871.5776	**4.4219**
Chlorophyll-a	C55H73MgN4O5	893.5431	893.5468	**4.0949**	893.5443	**1.2971**	893.5469	**4.2068**

Abbreviations: C-Hetero, *C. vulgaris* cultivated under heterotrophic conditions; DCM, dichloromethane:methanol (2:1, v/v) ; EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; Cer, ceramide; PI_Cer, inositolphosphoceramide; TG, triglyceride.

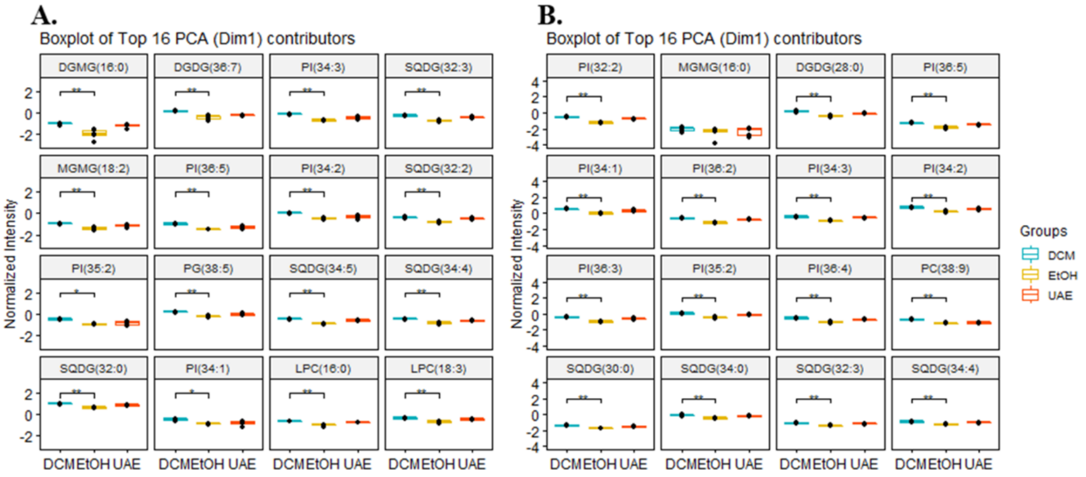


Figure S3.3. Box plots of the 16 main contributors for Dim1 using log normalized peak areas of all molecular ions of polar lipids identified in DCM, EtOH and UAE extracts from C-Auto (**A**) and from C-Hetero (**B**). Differences between solvent systems were determined by post hoc Dunn test, FDR adjusted, * $q < 0.05$, ** $q < 0.01$, *** $q < 0.001$. Abbreviations: C-Auto, *C. vulgaris* cultivated under autotrophic conditions; C-Hetero, *C. vulgaris* cultivated under heterotrophic conditions; DCM, dichloromethane:methanol (2:1, v/v); EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe; DGMG, digalactosylmonoacylglycerol; DGDG, digalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PG, phosphatidylglycerol; LPC, lysophosphatidylcholine; PI, phosphatidylinositol; PC, phosphatidylcholine.

Table S3.3. Results of Kruskal-Wallis test (FDR adjusted) using log normalized peak areas of all molecular ions of polar lipids identified in DCM, EtOH and UAE extracts from C-Auto.

	variable	.y.	n	statistic	df	p	method	p.adj
3	DGDG(32:0)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127
4	DGDG(32:1)	value	15	9.78	2	0.0075	Kruskal-Wallis	0.0127
5	DGDG(32:2)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
6	DGDG(32:3)	value	15	11.18	2	0.0037	Kruskal-Wallis	0.0127
7	DGDG(32:4)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
9	DGDG(34:1)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
11	DGDG(34:3)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0127
12	DGDG(34:4)	value	15	9.36	2	0.0093	Kruskal-Wallis	0.0127
13	DGDG(34:5)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
14	DGDG(34:6)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0127
16	DGDG(35:2)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
17	DGDG(35:3)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0127
18	DGDG(36:3)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127
19	DGDG(36:4)	value	15	10.58	2	0.0050	Kruskal-Wallis	0.0127
20	DGDG(36:5)	value	15	11.52	2	0.0032	Kruskal-Wallis	0.0127
21	DGDG(36:6)	value	15	9.74	2	0.0077	Kruskal-Wallis	0.0127

22	DGDG(36:7)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0127
23	DGMG(16:0)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
24	LPC(16:0)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
25	LPC(16:1)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
26	LPC(16:2)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0127
27	LPC(16:3)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
29	LPC(18:1)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
30	LPC(18:2)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
31	LPC(18:3)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
32	LPC(20:4)	value	15	11.18	2	0.0037	Kruskal-Wallis	0.0127
33	LPC(20:5)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
34	LPE(16:0)	value	15	10.64	2	0.0049	Kruskal-Wallis	0.0127
35	LPE(16:1)	value	15	11.06	2	0.0040	Kruskal-Wallis	0.0127
37	LPE(18:2)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0127
38	LPE(18:3)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0127
40	LPE(20:5)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127
42	MGDG(32:3)	value	15	9.78	2	0.0075	Kruskal-Wallis	0.0127
43	MGDG(32:4)	value	15	11.18	2	0.0037	Kruskal-Wallis	0.0127
44	MGDG(32:5)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
45	MGDG(32:6)	value	15	11.18	2	0.0037	Kruskal-Wallis	0.0127
46	MGDG(34:1)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0127
47	MGDG(34:2)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0127
49	MGDG(34:4)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
50	MGDG(34:5)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
51	MGDG(34:6)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
55	MGDG(36:4)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0127
56	MGDG(36:5)	value	15	10.26	2	0.0059	Kruskal-Wallis	0.0127
57	MGDG(36:6)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0127
59	MGMG(16:3)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0127
60	MGMG(18:2)	value	15	10.58	2	0.0050	Kruskal-Wallis	0.0127
61	MGMG(18:3)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
63	PC(32:0)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0127
64	PC(32:1)	value	15	9.78	2	0.0075	Kruskal-Wallis	0.0127
65	PC(32:2)	value	15	9.42	2	0.0090	Kruskal-Wallis	0.0127
66	PC(32:3)	value	15	9.38	2	0.0092	Kruskal-Wallis	0.0127
67	PC(32:4)	value	15	9.38	2	0.0092	Kruskal-Wallis	0.0127
70	PC(33:2)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127
71	PC(33:3)	value	15	9.38	2	0.0092	Kruskal-Wallis	0.0127
72	PC(34:1)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
73	PC(34:2)	value	15	9.98	2	0.0068	Kruskal-Wallis	0.0127
74	PC(34:3)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127
76	PC(34:5-OH)	value	15	9.62	2	0.0082	Kruskal-Wallis	0.0127
78	PC(34:6)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127
79	PC(34:7)	value	15	9.78	2	0.0075	Kruskal-Wallis	0.0127
80	PC(34:8)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127
81	PC(35:1)	value	15	9.42	2	0.0090	Kruskal-Wallis	0.0127
82	PC(35:2)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127

83	PC(35:3)	value	15	9.42	2	0.0090	Kruskal-Wallis	0.0127
84	PC(35:4)	value	15	9.98	2	0.0068	Kruskal-Wallis	0.0127
87	PC(36:3)	value	15	9.78	2	0.0075	Kruskal-Wallis	0.0127
88	PC(36:4-OH)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
90	PC(36:5)	value	15	9.42	2	0.0090	Kruskal-Wallis	0.0127
91	PC(36:6)	value	15	9.62	2	0.0082	Kruskal-Wallis	0.0127
96	PC(37:3)	value	15	9.78	2	0.0075	Kruskal-Wallis	0.0127
97	PC(37:4)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127
99	PC(38:2)	value	15	10.64	2	0.0049	Kruskal-Wallis	0.0127
100	PC(38:3)	value	15	9.62	2	0.0082	Kruskal-Wallis	0.0127
101	PC(38:4)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0127
113	PE(32:3)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
114	PE(32:4)	value	15	9.62	2	0.0082	Kruskal-Wallis	0.0127
118	PE(34:3)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127
120	PE(34:5)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0127
121	PE(34:6)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0127
122	PE(35:2)	value	15	9.98	2	0.0068	Kruskal-Wallis	0.0127
123	PE(35:3)	value	15	11.18	2	0.0037	Kruskal-Wallis	0.0127
124	PE(35:4)	value	15	9.36	2	0.0093	Kruskal-Wallis	0.0127
125	PE(36:2)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0127
126	PE(36:3)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
128	PE(36:4)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
129	PE(36:5)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0127
130	PE(36:6)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0127
132	PE(36:8)	value	15	9.98	2	0.0068	Kruskal-Wallis	0.0127
133	PE(36:9)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
134	PE(37:5)	value	15	9.38	2	0.0092	Kruskal-Wallis	0.0127
135	PE(38:7)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
136	PE(38:8)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0127
137	PE(38:9)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
138	PG(32:0)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0127
140	PG(33:0)	value	15	9.62	2	0.0082	Kruskal-Wallis	0.0127
141	PG(34:0)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0127
142	PG(34:1)	value	15	9.78	2	0.0075	Kruskal-Wallis	0.0127
143	PG(34:2)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0127
144	PG(34:3)	value	15	9.38	2	0.0092	Kruskal-Wallis	0.0127
145	PG(34:4)	value	15	9.42	2	0.0090	Kruskal-Wallis	0.0127
146	PG(36:0)	value	15	9.42	2	0.0090	Kruskal-Wallis	0.0127
147	PG(36:1)	value	15	9.38	2	0.0092	Kruskal-Wallis	0.0127
148	PG(36:2)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0127
149	PG(36:3)	value	15	9.38	2	0.0092	Kruskal-Wallis	0.0127
150	PG(36:4)	value	15	9.42	2	0.0090	Kruskal-Wallis	0.0127
151	PG(36:5)	value	15	9.78	2	0.0075	Kruskal-Wallis	0.0127
152	PG(38:5)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0127
153	PI(34:1)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0127
154	PI(34:2)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0127
155	PI(34:3)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0127

156	PI(35:2)	value	15	9.98	2	0.0068	Kruskal-Wallis	0.0127
157	PI(36:5)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
159	SQDG(32:0)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0127
160	SQDG(32:1)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0127
161	SQDG(32:2)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0127
162	SQDG(32:3)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
163	SQDG(34:0)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0127
164	SQDG(34:1)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0127
165	SQDG(34:2)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0127
166	SQDG(34:3)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0127
167	SQDG(34:4)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
168	SQDG(34:5)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
169	SQDG(36:3)	value	15	9.74	2	0.0077	Kruskal-Wallis	0.0127
170	SQDG(36:4)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0127
171	SQDG(36:5)	value	15	9.98	2	0.0068	Kruskal-Wallis	0.0127
172	SQDG(36:6)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0127
10	DGDG(34:2)	value	15	9.26	2	0.0098	Kruskal-Wallis	0.0132
77	PC(34:5)	value	15	8.96	2	0.0113	Kruskal-Wallis	0.0152
75	PC(34:4)	value	15	8.82	2	0.0122	Kruskal-Wallis	0.0161
89	PC(36:4)	value	15	8.82	2	0.0122	Kruskal-Wallis	0.0161
1	Cer(d35:0)	value	15	8.66	2	0.0132	Kruskal-Wallis	0.0171
15	DGDG(35:1)	value	15	8.64	2	0.0133	Kruskal-Wallis	0.0171
68	PC(32:5)	value	15	8.64	2	0.0133	Kruskal-Wallis	0.0171
98	PC(37:5)	value	15	8.66	2	0.0132	Kruskal-Wallis	0.0171
139	PG(32:1)	value	15	8.54	2	0.0140	Kruskal-Wallis	0.0178
58	MGMG(16:2)	value	15	8.24	2	0.0162	Kruskal-Wallis	0.0205
94	PC(36:9)	value	15	7.98	2	0.0185	Kruskal-Wallis	0.0231
95	PC(37:2)	value	15	7.98	2	0.0185	Kruskal-Wallis	0.0231
8	DGDG(32:5)	value	15	7.94	2	0.0189	Kruskal-Wallis	0.0234
48	MGDG(34:3)	value	15	7.76	2	0.0207	Kruskal-Wallis	0.0254
86	PC(36:2)	value	15	7.74	2	0.0209	Kruskal-Wallis	0.0255
85	PC(36:1)	value	15	7.62	2	0.0221	Kruskal-Wallis	0.0268
92	PC(36:7)	value	15	7.44	2	0.0242	Kruskal-Wallis	0.0291
69	PC(32:6)	value	15	7.28	2	0.0263	Kruskal-Wallis	0.0314
54	MGDG(36:3)	value	15	7.22	2	0.0271	Kruskal-Wallis	0.0321
111	PE(32:1)	value	15	6.86	2	0.0324	Kruskal-Wallis	0.0379
158	PI_Cer(t34:0-OH)	value	15	6.86	2	0.0324	Kruskal-Wallis	0.0379
41	MGDG(32:2)	value	15	6.74	2	0.0344	Kruskal-Wallis	0.0400
2	DGDG(28:0)	value	15	6.48	2	0.0392	Kruskal-Wallis	0.0447
28	LPC(18:0)	value	15	6.50	2	0.0388	Kruskal-Wallis	0.0447
115	PE(33:3)	value	15	6.48	2	0.0392	Kruskal-Wallis	0.0447
107	PE(30:0)	value	15	6.32	2	0.0424	Kruskal-Wallis	0.0480
131	PE(36:7)	value	15	6.26	2	0.0437	Kruskal-Wallis	0.0491
62	PC(30:3)	value	15	6.14	2	0.0464	Kruskal-Wallis	0.0518
93	PC(36:8)	value	15	6.02	2	0.0493	Kruskal-Wallis	0.0540
106	PC(38:9)	value	15	6.02	2	0.0493	Kruskal-Wallis	0.0540
108	PE(30:1)	value	15	6.02	2	0.0493	Kruskal-Wallis	0.0540

103	PC(38:6)	value	15	5.46	2	0.0652	Kruskal-Wallis	0.0710
127	PE(36:4-OH)	value	15	4.86	2	0.0880	Kruskal-Wallis	0.0952
116	PE(34:1)	value	15	4.74	2	0.0935	Kruskal-Wallis	0.1005
119	PE(34:4)	value	15	4.46	2	0.1080	Kruskal-Wallis	0.1154
36	LPE(18:1)	value	15	4.34	2	0.1140	Kruskal-Wallis	0.1203
104	PC(38:7)	value	15	4.34	2	0.1140	Kruskal-Wallis	0.1203
117	PE(34:2)	value	15	3.92	2	0.1410	Kruskal-Wallis	0.1479
39	LPE(20:4)	value	15	3.86	2	0.1450	Kruskal-Wallis	0.1512
102	PC(38:5)	value	15	3.62	2	0.1640	Kruskal-Wallis	0.1699
109	PE(30:2)	value	15	3.38	2	0.1850	Kruskal-Wallis	0.1905
105	PC(38:8)	value	15	3.26	2	0.1960	Kruskal-Wallis	0.2007
110	PE(30:3)	value	15	2.22	2	0.3300	Kruskal-Wallis	0.3359
53	MGDG(36:2)	value	15	1.62	2	0.4450	Kruskal-Wallis	0.4502
52	MGDG(35:1)	value	15	0.96	2	0.6190	Kruskal-Wallis	0.6226
112	PE(32:2)	value	15	0.06	2	0.9700	Kruskal-Wallis	0.9700

Abbreviations: C-Auto, *C. vulgaris* cultivated under autotrophic conditions; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; Cer, ceramide; PI_Cer, inositolphosphoceramide; TG, triglyceride.

Table S3.4. Results of Dunn's multiple comparison post-hoc test (FDR adjusted) using log normalized peak areas of significant molecular ions (*q* values < 0.05) of polar lipids identified in DCM, EtOH and UAE extracts from C-Auto.

	variable	.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
1	Cer(d35:0)	value	DCM	EtOH	5	5	-2.47	0.0133	0.0344	*
2	Cer(d35:0)	value	DCM	UAE	5	5	-2.62	0.0089	0.0283	*
3	Cer(d35:0)	value	EtOH	UAE	5	5	-0.14	0.8875	0.9053	ns
4	DGDG(28:0)	value	DCM	EtOH	5	5	1.27	0.2031	0.2568	ns
5	DGDG(28:0)	value	DCM	UAE	5	5	-1.27	0.2031	0.2568	ns
6	DGDG(28:0)	value	EtOH	UAE	5	5	-2.55	0.0109	0.0313	*
7	DGDG(32:0)	value	DCM	EtOH	5	5	-2.83	0.0047	0.0184	*
8	DGDG(32:0)	value	DCM	UAE	5	5	-2.47	0.0133	0.0344	*
9	DGDG(32:0)	value	EtOH	UAE	5	5	0.35	0.7237	0.7584	ns
10	DGDG(32:1)	value	DCM	EtOH	5	5	-2.97	0.0030	0.0138	*
11	DGDG(32:1)	value	DCM	UAE	5	5	-0.64	0.5245	0.5801	ns
12	DGDG(32:1)	value	EtOH	UAE	5	5	2.33	0.0196	0.0455	*
13	DGDG(32:2)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
14	DGDG(32:2)	value	DCM	UAE	5	5	-1.20	0.2293	0.2792	ns
15	DGDG(32:2)	value	EtOH	UAE	5	5	2.05	0.0403	0.0777	ns
16	DGDG(32:3)	value	DCM	EtOH	5	5	-3.32	0.0009	0.0083	**
17	DGDG(32:3)	value	DCM	UAE	5	5	-1.34	0.1791	0.2303	ns
18	DGDG(32:3)	value	EtOH	UAE	5	5	1.98	0.0477	0.0890	ns
19	DGDG(32:4)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
20	DGDG(32:4)	value	DCM	UAE	5	5	-2.05	0.0403	0.0777	ns
21	DGDG(32:4)	value	EtOH	UAE	5	5	1.20	0.2293	0.2792	ns
22	DGDG(32:5)	value	DCM	EtOH	5	5	-2.40	0.0162	0.0400	*
23	DGDG(32:5)	value	DCM	UAE	5	5	-2.47	0.0133	0.0344	*
24	DGDG(32:5)	value	EtOH	UAE	5	5	-0.07	0.9436	0.9478	ns

25	DGDG(34:1)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
26	DGDG(34:1)	value	DCM	UAE	5	5	-2.05	0.0403	0.0777	ns
27	DGDG(34:1)	value	EtOH	UAE	5	5	1.20	0.2293	0.2792	ns
28	DGDG(34:2)	value	DCM	EtOH	5	5	-3.04	0.0024	0.0122	*
29	DGDG(34:2)	value	DCM	UAE	5	5	-1.63	0.1039	0.1428	ns
30	DGDG(34:2)	value	EtOH	UAE	5	5	1.41	0.1573	0.2063	ns
31	DGDG(34:3)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0079	**
32	DGDG(34:3)	value	DCM	UAE	5	5	-1.91	0.0562	0.1004	ns
33	DGDG(34:3)	value	EtOH	UAE	5	5	1.48	0.1376	0.1820	ns
34	DGDG(34:4)	value	DCM	EtOH	5	5	-2.97	0.0030	0.0138	*
35	DGDG(34:4)	value	DCM	UAE	5	5	-0.85	0.3961	0.4569	ns
36	DGDG(34:4)	value	EtOH	UAE	5	5	2.12	0.0339	0.0698	ns
37	DGDG(34:5)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
38	DGDG(34:5)	value	DCM	UAE	5	5	-1.20	0.2293	0.2792	ns
39	DGDG(34:5)	value	EtOH	UAE	5	5	2.05	0.0403	0.0777	ns
40	DGDG(34:6)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0079	**
41	DGDG(34:6)	value	DCM	UAE	5	5	-1.91	0.0562	0.1004	ns
42	DGDG(34:6)	value	EtOH	UAE	5	5	1.48	0.1376	0.1820	ns
43	DGDG(35:1)	value	DCM	EtOH	5	5	-2.55	0.0109	0.0313	*
44	DGDG(35:1)	value	DCM	UAE	5	5	-2.55	0.0109	0.0313	*
45	DGDG(35:1)	value	EtOH	UAE	5	5	0.00	1.0000	1.0000	ns
46	DGDG(35:2)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
47	DGDG(35:2)	value	DCM	UAE	5	5	-2.05	0.0403	0.0777	ns
48	DGDG(35:2)	value	EtOH	UAE	5	5	1.20	0.2293	0.2792	ns
49	DGDG(35:3)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0095	**
50	DGDG(35:3)	value	DCM	UAE	5	5	-2.12	0.0339	0.0698	ns
51	DGDG(35:3)	value	EtOH	UAE	5	5	1.06	0.2888	0.3435	ns
52	DGDG(36:3)	value	DCM	EtOH	5	5	-2.83	0.0047	0.0184	*
53	DGDG(36:3)	value	DCM	UAE	5	5	-0.35	0.7237	0.7584	ns
54	DGDG(36:3)	value	EtOH	UAE	5	5	2.47	0.0133	0.0344	*
55	DGDG(36:4)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
56	DGDG(36:4)	value	DCM	UAE	5	5	-1.63	0.1039	0.1428	ns
57	DGDG(36:4)	value	EtOH	UAE	5	5	1.63	0.1039	0.1428	ns
58	DGDG(36:5)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0079	**
59	DGDG(36:5)	value	DCM	UAE	5	5	-1.70	0.0897	0.1290	ns
60	DGDG(36:5)	value	EtOH	UAE	5	5	1.70	0.0897	0.1290	ns
61	DGDG(36:6)	value	DCM	EtOH	5	5	-3.11	0.0019	0.0107	*
62	DGDG(36:6)	value	DCM	UAE	5	5	-1.34	0.1791	0.2303	ns
63	DGDG(36:6)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
64	DGDG(36:7)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0095	**
65	DGDG(36:7)	value	DCM	UAE	5	5	-2.12	0.0339	0.0698	ns
66	DGDG(36:7)	value	EtOH	UAE	5	5	1.06	0.2888	0.3435	ns
67	DGMG(16:0)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
68	DGMG(16:0)	value	DCM	UAE	5	5	-1.20	0.2293	0.2792	ns
69	DGMG(16:0)	value	EtOH	UAE	5	5	2.05	0.0403	0.0777	ns
70	LPC(16:0)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
71	LPC(16:0)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
72	LPC(16:0)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
73	LPC(16:1)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
74	LPC(16:1)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
75	LPC(16:1)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
76	LPC(16:2)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0079	**
77	LPC(16:2)	value	DCM	UAE	5	5	-1.63	0.1039	0.1428	ns

78	LPC(16:2)	value	EtOH	UAE	5	5	1.84	0.0660	0.1110	ns
79	LPC(16:3)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
80	LPC(16:3)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
81	LPC(16:3)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
82	LPC(18:0)	value	DCM	EtOH	5	5	-2.47	0.0133	0.0344	*
83	LPC(18:0)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
84	LPC(18:0)	value	EtOH	UAE	5	5	0.71	0.4795	0.5408	ns
85	LPC(18:1)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
86	LPC(18:1)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
87	LPC(18:1)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
88	LPC(18:2)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
89	LPC(18:2)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
90	LPC(18:2)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
91	LPC(18:3)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
92	LPC(18:3)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
93	LPC(18:3)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
94	LPC(20:4)	value	DCM	EtOH	5	5	-3.32	0.0009	0.0083	**
95	LPC(20:4)	value	DCM	UAE	5	5	-1.98	0.0477	0.0890	ns
96	LPC(20:4)	value	EtOH	UAE	5	5	1.34	0.1791	0.2303	ns
97	LPC(20:5)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
98	LPC(20:5)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
99	LPC(20:5)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
100	LPE(16:0)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
101	LPE(16:0)	value	DCM	UAE	5	5	-1.84	0.0660	0.1110	ns
102	LPE(16:0)	value	EtOH	UAE	5	5	1.41	0.1573	0.2063	ns
103	LPE(16:1)	value	DCM	EtOH	5	5	-3.32	0.0009	0.0083	**
104	LPE(16:1)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
105	LPE(16:1)	value	EtOH	UAE	5	5	1.56	0.1198	0.1636	ns
106	LPE(18:2)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0095	**
107	LPE(18:2)	value	DCM	UAE	5	5	-1.06	0.2888	0.3435	ns
108	LPE(18:2)	value	EtOH	UAE	5	5	2.12	0.0339	0.0698	ns
109	LPE(18:3)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0095	**
110	LPE(18:3)	value	DCM	UAE	5	5	-1.06	0.2888	0.3435	ns
111	LPE(18:3)	value	EtOH	UAE	5	5	2.12	0.0339	0.0698	ns
112	LPE(20:5)	value	DCM	EtOH	5	5	-2.83	0.0047	0.0184	*
113	LPE(20:5)	value	DCM	UAE	5	5	-0.35	0.7237	0.7584	ns
114	LPE(20:5)	value	EtOH	UAE	5	5	2.47	0.0133	0.0344	*
115	MGDG(32:2)	value	DCM	EtOH	5	5	-2.05	0.0403	0.0777	ns
116	MGDG(32:2)	value	DCM	UAE	5	5	0.35	0.7237	0.7584	ns
117	MGDG(32:2)	value	EtOH	UAE	5	5	2.40	0.0162	0.0400	*
118	MGDG(32:3)	value	DCM	EtOH	5	5	-2.97	0.0030	0.0138	*
119	MGDG(32:3)	value	DCM	UAE	5	5	-2.33	0.0196	0.0455	*
120	MGDG(32:3)	value	EtOH	UAE	5	5	0.64	0.5245	0.5801	ns
121	MGDG(32:4)	value	DCM	EtOH	5	5	-3.32	0.0009	0.0083	**
122	MGDG(32:4)	value	DCM	UAE	5	5	-1.98	0.0477	0.0890	ns
123	MGDG(32:4)	value	EtOH	UAE	5	5	1.34	0.1791	0.2303	ns
124	MGDG(32:5)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
125	MGDG(32:5)	value	DCM	UAE	5	5	-2.05	0.0403	0.0777	ns
126	MGDG(32:5)	value	EtOH	UAE	5	5	1.20	0.2293	0.2792	ns
127	MGDG(32:6)	value	DCM	EtOH	5	5	-3.32	0.0009	0.0083	**
128	MGDG(32:6)	value	DCM	UAE	5	5	-1.98	0.0477	0.0890	ns
129	MGDG(32:6)	value	EtOH	UAE	5	5	1.34	0.1791	0.2303	ns
130	MGDG(34:1)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0079	**

131	MGDG(34:1)	value	DCM	UAE	5	5	-1.91	0.0562	0.1004	ns
132	MGDG(34:1)	value	EtOH	UAE	5	5	1.48	0.1376	0.1820	ns
133	MGDG(34:2)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0079	**
134	MGDG(34:2)	value	DCM	UAE	5	5	-1.91	0.0562	0.1004	ns
135	MGDG(34:2)	value	EtOH	UAE	5	5	1.48	0.1376	0.1820	ns
136	MGDG(34:3)	value	DCM	EtOH	5	5	-2.69	0.0072	0.0245	*
137	MGDG(34:3)	value	DCM	UAE	5	5	-0.71	0.4795	0.5408	ns
138	MGDG(34:3)	value	EtOH	UAE	5	5	1.98	0.0477	0.0890	ns
139	MGDG(34:4)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
140	MGDG(34:4)	value	DCM	UAE	5	5	-2.05	0.0403	0.0777	ns
141	MGDG(34:4)	value	EtOH	UAE	5	5	1.20	0.2293	0.2792	ns
142	MGDG(34:5)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
143	MGDG(34:5)	value	DCM	UAE	5	5	-2.05	0.0403	0.0777	ns
144	MGDG(34:5)	value	EtOH	UAE	5	5	1.20	0.2293	0.2792	ns
145	MGDG(34:6)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
146	MGDG(34:6)	value	DCM	UAE	5	5	-2.05	0.0403	0.0777	ns
147	MGDG(34:6)	value	EtOH	UAE	5	5	1.20	0.2293	0.2792	ns
148	MGDG(36:3)	value	DCM	EtOH	5	5	2.62	0.0089	0.0283	*
149	MGDG(36:3)	value	DCM	UAE	5	5	0.78	0.4367	0.4949	ns
150	MGDG(36:3)	value	EtOH	UAE	5	5	-1.84	0.0660	0.1110	ns
151	MGDG(36:4)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0079	**
152	MGDG(36:4)	value	DCM	UAE	5	5	-1.84	0.0660	0.1110	ns
153	MGDG(36:4)	value	EtOH	UAE	5	5	1.63	0.1039	0.1428	ns
154	MGDG(36:5)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0095	**
155	MGDG(36:5)	value	DCM	UAE	5	5	-1.91	0.0562	0.1004	ns
156	MGDG(36:5)	value	EtOH	UAE	5	5	1.27	0.2031	0.2568	ns
157	MGDG(36:6)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0095	**
158	MGDG(36:6)	value	DCM	UAE	5	5	-2.12	0.0339	0.0698	ns
159	MGDG(36:6)	value	EtOH	UAE	5	5	1.06	0.2888	0.3435	ns
160	MGMG(16:2)	value	DCM	EtOH	5	5	-2.83	0.0047	0.0184	*
161	MGMG(16:2)	value	DCM	UAE	5	5	-1.84	0.0660	0.1110	ns
162	MGMG(16:2)	value	EtOH	UAE	5	5	0.99	0.3222	0.3812	ns
163	MGMG(16:3)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0079	**
164	MGMG(16:3)	value	DCM	UAE	5	5	-1.84	0.0660	0.1110	ns
165	MGMG(16:3)	value	EtOH	UAE	5	5	1.63	0.1039	0.1428	ns
166	MGMG(18:2)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0083	**
167	MGMG(18:2)	value	DCM	UAE	5	5	-1.63	0.1039	0.1428	ns
168	MGMG(18:2)	value	EtOH	UAE	5	5	1.63	0.1039	0.1428	ns
169	MGMG(18:3)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
170	MGMG(18:3)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
171	MGMG(18:3)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
172	PC(32:0)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0079	**
173	PC(32:0)	value	DCM	UAE	5	5	-1.84	0.0660	0.1110	ns
174	PC(32:0)	value	EtOH	UAE	5	5	1.63	0.1039	0.1428	ns
175	PC(32:1)	value	DCM	EtOH	5	5	-2.33	0.0196	0.0455	*
176	PC(32:1)	value	DCM	UAE	5	5	0.64	0.5245	0.5801	ns
177	PC(32:1)	value	EtOH	UAE	5	5	2.97	0.0030	0.0138	*
178	PC(32:2)	value	DCM	EtOH	5	5	-2.55	0.0109	0.0313	*
179	PC(32:2)	value	DCM	UAE	5	5	0.21	0.8320	0.8543	ns
180	PC(32:2)	value	EtOH	UAE	5	5	2.76	0.0058	0.0209	*
181	PC(32:3)	value	DCM	EtOH	5	5	-2.62	0.0089	0.0283	*
182	PC(32:3)	value	DCM	UAE	5	5	0.07	0.9436	0.9478	ns
183	PC(32:3)	value	EtOH	UAE	5	5	2.69	0.0072	0.0245	*

184	PC(32:4)	value	DCM	EtOH	5	5	-2.62	0.0089	0.0283	*
185	PC(32:4)	value	DCM	UAE	5	5	0.07	0.9436	0.9478	ns
186	PC(32:4)	value	EtOH	UAE	5	5	2.69	0.0072	0.0245	*
187	PC(32:5)	value	DCM	EtOH	5	5	-2.55	0.0109	0.0313	*
188	PC(32:5)	value	DCM	UAE	5	5	0.00	1.0000	1.0000	ns
189	PC(32:5)	value	EtOH	UAE	5	5	2.55	0.0109	0.0313	*
190	PC(32:6)	value	DCM	EtOH	5	5	-2.40	0.0162	0.0400	*
191	PC(32:6)	value	DCM	UAE	5	5	-0.14	0.8875	0.9053	ns
192	PC(32:6)	value	EtOH	UAE	5	5	2.26	0.0237	0.0527	ns
193	PC(33:2)	value	DCM	EtOH	5	5	-2.47	0.0133	0.0344	*
194	PC(33:2)	value	DCM	UAE	5	5	0.35	0.7237	0.7584	ns
195	PC(33:2)	value	EtOH	UAE	5	5	2.83	0.0047	0.0184	*
196	PC(33:3)	value	DCM	EtOH	5	5	-2.62	0.0089	0.0283	*
197	PC(33:3)	value	DCM	UAE	5	5	0.07	0.9436	0.9478	ns
198	PC(33:3)	value	EtOH	UAE	5	5	2.69	0.0072	0.0245	*
199	PC(34:1)	value	DCM	EtOH	5	5	-2.05	0.0403	0.0777	ns
200	PC(34:1)	value	DCM	UAE	5	5	1.20	0.2293	0.2792	ns
201	PC(34:1)	value	EtOH	UAE	5	5	3.25	0.0011	0.0083	**
202	PC(34:2)	value	DCM	EtOH	5	5	-2.26	0.0237	0.0527	ns
203	PC(34:2)	value	DCM	UAE	5	5	0.78	0.4367	0.4949	ns
204	PC(34:2)	value	EtOH	UAE	5	5	3.04	0.0024	0.0122	*
205	PC(34:3)	value	DCM	EtOH	5	5	-2.83	0.0047	0.0184	*
206	PC(34:3)	value	DCM	UAE	5	5	-0.35	0.7237	0.7584	ns
207	PC(34:3)	value	EtOH	UAE	5	5	2.47	0.0133	0.0344	*
208	PC(34:4)	value	DCM	EtOH	5	5	-2.33	0.0196	0.0455	*
209	PC(34:4)	value	DCM	UAE	5	5	0.42	0.6714	0.7234	ns
210	PC(34:4)	value	EtOH	UAE	5	5	2.76	0.0058	0.0209	*
211	PC(34:5-OH)	value	DCM	EtOH	5	5	-2.40	0.0162	0.0400	*
212	PC(34:5-OH)	value	DCM	UAE	5	5	0.49	0.6206	0.6734	ns
213	PC(34:5-OH)	value	EtOH	UAE	5	5	2.90	0.0037	0.0165	*
214	PC(34:5)	value	DCM	EtOH	5	5	-2.26	0.0237	0.0527	ns
215	PC(34:5)	value	DCM	UAE	5	5	0.57	0.5716	0.6292	ns
216	PC(34:5)	value	EtOH	UAE	5	5	2.83	0.0047	0.0184	*
217	PC(34:6)	value	DCM	EtOH	5	5	-2.83	0.0047	0.0184	*
218	PC(34:6)	value	DCM	UAE	5	5	-0.35	0.7237	0.7584	ns
219	PC(34:6)	value	EtOH	UAE	5	5	2.47	0.0133	0.0344	*
220	PC(34:7)	value	DCM	EtOH	5	5	-2.33	0.0196	0.0455	*
221	PC(34:7)	value	DCM	UAE	5	5	0.64	0.5245	0.5801	ns
222	PC(34:7)	value	EtOH	UAE	5	5	2.97	0.0030	0.0138	*
223	PC(34:8)	value	DCM	EtOH	5	5	-2.83	0.0047	0.0184	*
224	PC(34:8)	value	DCM	UAE	5	5	-0.35	0.7237	0.7584	ns
225	PC(34:8)	value	EtOH	UAE	5	5	2.47	0.0133	0.0344	*
226	PC(35:1)	value	DCM	EtOH	5	5	-2.55	0.0109	0.0313	*
227	PC(35:1)	value	DCM	UAE	5	5	0.21	0.8320	0.8543	ns
228	PC(35:1)	value	EtOH	UAE	5	5	2.76	0.0058	0.0209	*
229	PC(35:2)	value	DCM	EtOH	5	5	-2.47	0.0133	0.0344	*
230	PC(35:2)	value	DCM	UAE	5	5	0.35	0.7237	0.7584	ns
231	PC(35:2)	value	EtOH	UAE	5	5	2.83	0.0047	0.0184	*
232	PC(35:3)	value	DCM	EtOH	5	5	-2.55	0.0109	0.0313	*
233	PC(35:3)	value	DCM	UAE	5	5	0.21	0.8320	0.8543	ns
234	PC(35:3)	value	EtOH	UAE	5	5	2.76	0.0058	0.0209	*
235	PC(35:4)	value	DCM	EtOH	5	5	-2.26	0.0237	0.0527	ns
236	PC(35:4)	value	DCM	UAE	5	5	0.78	0.4367	0.4949	ns

237	PC(35:4)	value	EtOH	UAE	5	5	3.04	0.0024	0.0122	*
238	PC(36:1)	value	DCM	EtOH	5	5	-1.27	0.2031	0.2568	ns
239	PC(36:1)	value	DCM	UAE	5	5	1.48	0.1376	0.1820	ns
240	PC(36:1)	value	EtOH	UAE	5	5	2.76	0.0058	0.0209	*
241	PC(36:2)	value	DCM	EtOH	5	5	-2.76	0.0058	0.0209	*
242	PC(36:2)	value	DCM	UAE	5	5	-1.06	0.2888	0.3435	ns
243	PC(36:2)	value	EtOH	UAE	5	5	1.70	0.0897	0.1290	ns
244	PC(36:3)	value	DCM	EtOH	5	5	-2.33	0.0196	0.0455	*
245	PC(36:3)	value	DCM	UAE	5	5	0.64	0.5245	0.5801	ns
246	PC(36:3)	value	EtOH	UAE	5	5	2.97	0.0030	0.0138	*
247	PC(36:4-OH)	value	DCM	EtOH	5	5	-1.77	0.0771	0.1120	ns
248	PC(36:4-OH)	value	DCM	UAE	5	5	1.77	0.0771	0.1120	ns
249	PC(36:4-OH)	value	EtOH	UAE	5	5	3.54	0.0004	0.0079	**
250	PC(36:4)	value	DCM	EtOH	5	5	-2.33	0.0196	0.0455	*
251	PC(36:4)	value	DCM	UAE	5	5	0.42	0.6714	0.7234	ns
252	PC(36:4)	value	EtOH	UAE	5	5	2.76	0.0058	0.0209	*
253	PC(36:5)	value	DCM	EtOH	5	5	-2.55	0.0109	0.0313	*
254	PC(36:5)	value	DCM	UAE	5	5	0.21	0.8320	0.8543	ns
255	PC(36:5)	value	EtOH	UAE	5	5	2.76	0.0058	0.0209	*
256	PC(36:6)	value	DCM	EtOH	5	5	-2.90	0.0037	0.0165	*
257	PC(36:6)	value	DCM	UAE	5	5	-0.49	0.6206	0.6734	ns
258	PC(36:6)	value	EtOH	UAE	5	5	2.40	0.0162	0.0400	*
259	PC(36:7)	value	DCM	EtOH	5	5	-2.12	0.0339	0.0698	ns
260	PC(36:7)	value	DCM	UAE	5	5	0.42	0.6714	0.7234	ns
261	PC(36:7)	value	EtOH	UAE	5	5	2.55	0.0109	0.0313	*
262	PC(36:9)	value	DCM	EtOH	5	5	-2.55	0.0109	0.0313	*
263	PC(36:9)	value	DCM	UAE	5	5	-0.21	0.8320	0.8543	ns
264	PC(36:9)	value	EtOH	UAE	5	5	2.33	0.0196	0.0455	*
265	PC(37:2)	value	DCM	EtOH	5	5	-2.55	0.0109	0.0313	*
266	PC(37:2)	value	DCM	UAE	5	5	-0.21	0.8320	0.8543	ns
267	PC(37:2)	value	EtOH	UAE	5	5	2.33	0.0196	0.0455	*
268	PC(37:3)	value	DCM	EtOH	5	5	-2.33	0.0196	0.0455	*
269	PC(37:3)	value	DCM	UAE	5	5	0.64	0.5245	0.5801	ns
270	PC(37:3)	value	EtOH	UAE	5	5	2.97	0.0030	0.0138	*
271	PC(37:4)	value	DCM	EtOH	5	5	-2.47	0.0133	0.0344	*
272	PC(37:4)	value	DCM	UAE	5	5	0.35	0.7237	0.7584	ns
273	PC(37:4)	value	EtOH	UAE	5	5	2.83	0.0047	0.0184	*
274	PC(37:5)	value	DCM	EtOH	5	5	-2.47	0.0133	0.0344	*
275	PC(37:5)	value	DCM	UAE	5	5	0.14	0.8875	0.9053	ns
276	PC(37:5)	value	EtOH	UAE	5	5	2.62	0.0089	0.0283	*
277	PC(38:2)	value	DCM	EtOH	5	5	-1.84	0.0660	0.1110	ns
278	PC(38:2)	value	DCM	UAE	5	5	1.41	0.1573	0.2063	ns
279	PC(38:2)	value	EtOH	UAE	5	5	3.25	0.0011	0.0083	**
280	PC(38:3)	value	DCM	EtOH	5	5	-2.05	0.0403	0.0777	ns
281	PC(38:3)	value	DCM	UAE	5	5	0.99	0.3222	0.3812	ns
282	PC(38:3)	value	EtOH	UAE	5	5	3.04	0.0024	0.0122	*
283	PC(38:4)	value	DCM	EtOH	5	5	-2.19	0.0284	0.0612	ns
284	PC(38:4)	value	DCM	UAE	5	5	0.92	0.3580	0.4149	ns
285	PC(38:4)	value	EtOH	UAE	5	5	3.11	0.0019	0.0107	*
286	PE(30:0)	value	DCM	EtOH	5	5	-0.57	0.5716	0.6292	ns
287	PE(30:0)	value	DCM	UAE	5	5	-2.40	0.0162	0.0400	*
288	PE(30:0)	value	EtOH	UAE	5	5	-1.84	0.0660	0.1110	ns
289	PE(32:1)	value	DCM	EtOH	5	5	0.49	0.6206	0.6734	ns

290	PE(32:1)	value	DCM	UAE	5	5	-1.98	0.0477	0.0890	ns
291	PE(32:1)	value	EtOH	UAE	5	5	-2.47	0.0133	0.0344	*
292	PE(32:3)	value	DCM	EtOH	5	5	-2.05	0.0403	0.0777	ns
293	PE(32:3)	value	DCM	UAE	5	5	1.20	0.2293	0.2792	ns
294	PE(32:3)	value	EtOH	UAE	5	5	3.25	0.0011	0.0083	**
295	PE(32:4)	value	DCM	EtOH	5	5	-2.90	0.0037	0.0165	*
296	PE(32:4)	value	DCM	UAE	5	5	-0.49	0.6206	0.6734	ns
297	PE(32:4)	value	EtOH	UAE	5	5	2.40	0.0162	0.0400	*
298	PE(33:3)	value	DCM	EtOH	5	5	-2.55	0.0109	0.0313	*
299	PE(33:3)	value	DCM	UAE	5	5	-1.27	0.2031	0.2568	ns
300	PE(33:3)	value	EtOH	UAE	5	5	1.27	0.2031	0.2568	ns
301	PE(34:3)	value	DCM	EtOH	5	5	-2.47	0.0133	0.0344	*
302	PE(34:3)	value	DCM	UAE	5	5	0.35	0.7237	0.7584	ns
303	PE(34:3)	value	EtOH	UAE	5	5	2.83	0.0047	0.0184	*
304	PE(34:5)	value	DCM	EtOH	5	5	-2.47	0.0133	0.0344	*
305	PE(34:5)	value	DCM	UAE	5	5	0.35	0.7237	0.7584	ns
306	PE(34:5)	value	EtOH	UAE	5	5	2.83	0.0047	0.0184	*
307	PE(34:6)	value	DCM	EtOH	5	5	-2.05	0.0403	0.0777	ns
308	PE(34:6)	value	DCM	UAE	5	5	1.20	0.2293	0.2792	ns
309	PE(34:6)	value	EtOH	UAE	5	5	3.25	0.0011	0.0083	**
310	PE(35:2)	value	DCM	EtOH	5	5	-3.04	0.0024	0.0122	*
311	PE(35:2)	value	DCM	UAE	5	5	-0.78	0.4367	0.4949	ns
312	PE(35:2)	value	EtOH	UAE	5	5	2.26	0.0237	0.0527	ns
313	PE(35:3)	value	DCM	EtOH	5	5	-1.98	0.0477	0.0890	ns
314	PE(35:3)	value	DCM	UAE	5	5	1.34	0.1791	0.2303	ns
315	PE(35:3)	value	EtOH	UAE	5	5	3.32	0.0009	0.0083	**
316	PE(35:4)	value	DCM	EtOH	5	5	-2.97	0.0030	0.0138	*
317	PE(35:4)	value	DCM	UAE	5	5	-2.12	0.0339	0.0698	ns
318	PE(35:4)	value	EtOH	UAE	5	5	0.85	0.3961	0.4569	ns
319	PE(36:2)	value	DCM	EtOH	5	5	-3.11	0.0019	0.0107	*
320	PE(36:2)	value	DCM	UAE	5	5	-2.19	0.0284	0.0612	ns
321	PE(36:2)	value	EtOH	UAE	5	5	0.92	0.3580	0.4149	ns
322	PE(36:3)	value	DCM	EtOH	5	5	-1.77	0.0771	0.1120	ns
323	PE(36:3)	value	DCM	UAE	5	5	1.77	0.0771	0.1120	ns
324	PE(36:3)	value	EtOH	UAE	5	5	3.54	0.0004	0.0079	**
325	PE(36:4)	value	DCM	EtOH	5	5	-1.77	0.0771	0.1120	ns
326	PE(36:4)	value	DCM	UAE	5	5	1.77	0.0771	0.1120	ns
327	PE(36:4)	value	EtOH	UAE	5	5	3.54	0.0004	0.0079	**
328	PE(36:5)	value	DCM	EtOH	5	5	-2.19	0.0284	0.0612	ns
329	PE(36:5)	value	DCM	UAE	5	5	0.92	0.3580	0.4149	ns
330	PE(36:5)	value	EtOH	UAE	5	5	3.11	0.0019	0.0107	*
331	PE(36:6)	value	DCM	EtOH	5	5	-1.91	0.0562	0.1004	ns
332	PE(36:6)	value	DCM	UAE	5	5	1.48	0.1376	0.1820	ns
333	PE(36:6)	value	EtOH	UAE	5	5	3.39	0.0007	0.0079	**
334	PE(36:7)	value	DCM	EtOH	5	5	-0.92	0.3580	0.4149	ns
335	PE(36:7)	value	DCM	UAE	5	5	1.56	0.1198	0.1636	ns
336	PE(36:7)	value	EtOH	UAE	5	5	2.47	0.0133	0.0344	*
337	PE(36:8)	value	DCM	EtOH	5	5	0.78	0.4367	0.4949	ns
338	PE(36:8)	value	DCM	UAE	5	5	3.04	0.0024	0.0122	*
339	PE(36:8)	value	EtOH	UAE	5	5	2.26	0.0237	0.0527	ns
340	PE(36:9)	value	DCM	EtOH	5	5	-1.77	0.0771	0.1120	ns
341	PE(36:9)	value	DCM	UAE	5	5	1.77	0.0771	0.1120	ns
342	PE(36:9)	value	EtOH	UAE	5	5	3.54	0.0004	0.0079	**

343	PE(37:5)	value	DCM	EtOH	5	5	-1.20	0.2293	0.2792	ns
344	PE(37:5)	value	DCM	UAE	5	5	1.84	0.0660	0.1110	ns
345	PE(37:5)	value	EtOH	UAE	5	5	3.04	0.0024	0.0122	*
346	PE(38:7)	value	DCM	EtOH	5	5	-1.77	0.0771	0.1120	ns
347	PE(38:7)	value	DCM	UAE	5	5	1.77	0.0771	0.1120	ns
348	PE(38:7)	value	EtOH	UAE	5	5	3.54	0.0004	0.0079	**
349	PE(38:8)	value	DCM	EtOH	5	5	-1.06	0.2888	0.3435	ns
350	PE(38:8)	value	DCM	UAE	5	5	2.12	0.0339	0.0698	ns
351	PE(38:8)	value	EtOH	UAE	5	5	3.18	0.0015	0.0095	**
352	PE(38:9)	value	DCM	EtOH	5	5	-1.77	0.0771	0.1120	ns
353	PE(38:9)	value	DCM	UAE	5	5	1.77	0.0771	0.1120	ns
354	PE(38:9)	value	EtOH	UAE	5	5	3.54	0.0004	0.0079	**
355	PG(32:0)	value	DCM	EtOH	5	5	-3.11	0.0019	0.0107	*
356	PG(32:0)	value	DCM	UAE	5	5	-0.92	0.3580	0.4149	ns
357	PG(32:0)	value	EtOH	UAE	5	5	2.19	0.0284	0.0612	ns
358	PG(32:1)	value	DCM	EtOH	5	5	-2.90	0.0037	0.0165	*
359	PG(32:1)	value	DCM	UAE	5	5	-1.13	0.2579	0.3132	ns
360	PG(32:1)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
361	PG(33:0)	value	DCM	EtOH	5	5	-2.90	0.0037	0.0165	*
362	PG(33:0)	value	DCM	UAE	5	5	-0.49	0.6206	0.6734	ns
363	PG(33:0)	value	EtOH	UAE	5	5	2.40	0.0162	0.0400	*
364	PG(34:0)	value	DCM	EtOH	5	5	-2.19	0.0284	0.0612	ns
365	PG(34:0)	value	DCM	UAE	5	5	0.92	0.3580	0.4149	ns
366	PG(34:0)	value	EtOH	UAE	5	5	3.11	0.0019	0.0107	*
367	PG(34:1)	value	DCM	EtOH	5	5	-2.33	0.0196	0.0455	*
368	PG(34:1)	value	DCM	UAE	5	5	0.64	0.5245	0.5801	ns
369	PG(34:1)	value	EtOH	UAE	5	5	2.97	0.0030	0.0138	*
370	PG(34:2)	value	DCM	EtOH	5	5	-2.19	0.0284	0.0612	ns
371	PG(34:2)	value	DCM	UAE	5	5	0.92	0.3580	0.4149	ns
372	PG(34:2)	value	EtOH	UAE	5	5	3.11	0.0019	0.0107	*
373	PG(34:3)	value	DCM	EtOH	5	5	-2.62	0.0089	0.0283	*
374	PG(34:3)	value	DCM	UAE	5	5	0.07	0.9436	0.9478	ns
375	PG(34:3)	value	EtOH	UAE	5	5	2.69	0.0072	0.0245	*
376	PG(34:4)	value	DCM	EtOH	5	5	-2.76	0.0058	0.0209	*
377	PG(34:4)	value	DCM	UAE	5	5	-0.21	0.8320	0.8543	ns
378	PG(34:4)	value	EtOH	UAE	5	5	2.55	0.0109	0.0313	*
379	PG(36:0)	value	DCM	EtOH	5	5	-2.76	0.0058	0.0209	*
380	PG(36:0)	value	DCM	UAE	5	5	-0.21	0.8320	0.8543	ns
381	PG(36:0)	value	EtOH	UAE	5	5	2.55	0.0109	0.0313	*
382	PG(36:1)	value	DCM	EtOH	5	5	-2.62	0.0089	0.0283	*
383	PG(36:1)	value	DCM	UAE	5	5	0.07	0.9436	0.9478	ns
384	PG(36:1)	value	EtOH	UAE	5	5	2.69	0.0072	0.0245	*
385	PG(36:2)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0079	**
386	PG(36:2)	value	DCM	UAE	5	5	-1.91	0.0562	0.1004	ns
387	PG(36:2)	value	EtOH	UAE	5	5	1.48	0.1376	0.1820	ns
388	PG(36:3)	value	DCM	EtOH	5	5	-2.69	0.0072	0.0245	*
389	PG(36:3)	value	DCM	UAE	5	5	-0.07	0.9436	0.9478	ns
390	PG(36:3)	value	EtOH	UAE	5	5	2.62	0.0089	0.0283	*
391	PG(36:4)	value	DCM	EtOH	5	5	-2.76	0.0058	0.0209	*
392	PG(36:4)	value	DCM	UAE	5	5	-0.21	0.8320	0.8543	ns
393	PG(36:4)	value	EtOH	UAE	5	5	2.55	0.0109	0.0313	*
394	PG(36:5)	value	DCM	EtOH	5	5	-2.97	0.0030	0.0138	*
395	PG(36:5)	value	DCM	UAE	5	5	-0.64	0.5245	0.5801	ns

396	PG(36:5)	value	EtOH	UAE	5	5	2.33	0.0196	0.0455	*
397	PG(38:5)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0079	**
398	PG(38:5)	value	DCM	UAE	5	5	-1.63	0.1039	0.1428	ns
399	PG(38:5)	value	EtOH	UAE	5	5	1.84	0.0660	0.1110	ns
400	PI(34:1)	value	DCM	EtOH	5	5	-3.11	0.0019	0.0107	*
401	PI(34:1)	value	DCM	UAE	5	5	-2.19	0.0284	0.0612	ns
402	PI(34:1)	value	EtOH	UAE	5	5	0.92	0.3580	0.4149	ns
403	PI(34:2)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0095	**
404	PI(34:2)	value	DCM	UAE	5	5	-2.12	0.0339	0.0698	ns
405	PI(34:2)	value	EtOH	UAE	5	5	1.06	0.2888	0.3435	ns
406	PI(34:3)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0079	**
407	PI(34:3)	value	DCM	UAE	5	5	-1.91	0.0562	0.1004	ns
408	PI(34:3)	value	EtOH	UAE	5	5	1.48	0.1376	0.1820	ns
409	PI(35:2)	value	DCM	EtOH	5	5	-3.04	0.0024	0.0122	*
410	PI(35:2)	value	DCM	UAE	5	5	-2.26	0.0237	0.0527	ns
411	PI(35:2)	value	EtOH	UAE	5	5	0.78	0.4367	0.4949	ns
412	PI(36:5)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
413	PI(36:5)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
414	PI(36:5)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
415	PI_Cer(t34:0-OH)	value	DCM	EtOH	5	5	0.49	0.6206	0.6734	ns
416	PI_Cer(t34:0-OH)	value	DCM	UAE	5	5	2.47	0.0133	0.0344	*
417	PI_Cer(t34:0-OH)	value	EtOH	UAE	5	5	1.98	0.0477	0.0890	ns
418	SQDG(32:0)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0079	**
419	SQDG(32:0)	value	DCM	UAE	5	5	-1.63	0.1039	0.1428	ns
420	SQDG(32:0)	value	EtOH	UAE	5	5	1.84	0.0660	0.1110	ns
421	SQDG(32:1)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0079	**
422	SQDG(32:1)	value	DCM	UAE	5	5	-1.63	0.1039	0.1428	ns
423	SQDG(32:1)	value	EtOH	UAE	5	5	1.84	0.0660	0.1110	ns
424	SQDG(32:2)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0079	**
425	SQDG(32:2)	value	DCM	UAE	5	5	-1.63	0.1039	0.1428	ns
426	SQDG(32:2)	value	EtOH	UAE	5	5	1.84	0.0660	0.1110	ns
427	SQDG(32:3)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
428	SQDG(32:3)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
429	SQDG(32:3)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
430	SQDG(34:0)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0079	**
431	SQDG(34:0)	value	DCM	UAE	5	5	-1.48	0.1376	0.1820	ns
432	SQDG(34:0)	value	EtOH	UAE	5	5	1.91	0.0562	0.1004	ns
433	SQDG(34:1)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0079	**
434	SQDG(34:1)	value	DCM	UAE	5	5	-1.63	0.1039	0.1428	ns
435	SQDG(34:1)	value	EtOH	UAE	5	5	1.84	0.0660	0.1110	ns
436	SQDG(34:2)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0079	**
437	SQDG(34:2)	value	DCM	UAE	5	5	-1.48	0.1376	0.1820	ns
438	SQDG(34:2)	value	EtOH	UAE	5	5	1.91	0.0562	0.1004	ns
439	SQDG(34:3)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0079	**
440	SQDG(34:3)	value	DCM	UAE	5	5	-1.63	0.1039	0.1428	ns
441	SQDG(34:3)	value	EtOH	UAE	5	5	1.84	0.0660	0.1110	ns
442	SQDG(34:4)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
443	SQDG(34:4)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
444	SQDG(34:4)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
445	SQDG(34:5)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
446	SQDG(34:5)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
447	SQDG(34:5)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
448	SQDG(36:3)	value	DCM	EtOH	5	5	-3.11	0.0019	0.0107	*

449	SQDG(36:3)	value	DCM	UAE	5	5	-1.34	0.1791	0.2303	ns
450	SQDG(36:3)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns
451	SQDG(36:4)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0079	**
452	SQDG(36:4)	value	DCM	UAE	5	5	-1.48	0.1376	0.1820	ns
453	SQDG(36:4)	value	EtOH	UAE	5	5	1.91	0.0562	0.1004	ns
454	SQDG(36:5)	value	DCM	EtOH	5	5	-3.04	0.0024	0.0122	*
455	SQDG(36:5)	value	DCM	UAE	5	5	-0.78	0.4367	0.4949	ns
456	SQDG(36:5)	value	EtOH	UAE	5	5	2.26	0.0237	0.0527	ns
457	SQDG(36:6)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0079	**
458	SQDG(36:6)	value	DCM	UAE	5	5	-1.77	0.0771	0.1120	ns
459	SQDG(36:6)	value	EtOH	UAE	5	5	1.77	0.0771	0.1120	ns

Abbreviations: C-Auto, *C. vulgaris* cultivated under autotrophic conditions; DCM, dichloromethane:methanol (2:1, v/v) ; EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; Cer, ceramide; PI_Cer, inositolphosphoceramide; TG, triglyceride.

Table S3.5. Results of Kruskal-Wallis test (FDR adjusted) using log normalized peak areas of all molecular ions of polar lipids identified in DCM, EtOH and UAE extracts from C-Hetero.

	variable	.y.	n	statistic	df	p	method	p.adj
2	DGDG(28:0)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0292
143	PG(38:5)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0292
144	PI(32:2)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0292
145	PI(34:1)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0292
146	PI(34:2)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0292
147	PI(34:3)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0292
149	PI(36:2)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0292
150	PI(36:3)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0292
151	PI(36:4)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0292
152	PI(36:5)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0292
155	SQDG(32:0)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0292
160	SQDG(34:1)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0292
161	SQDG(34:2)	value	15	12.02	2	0.0025	Kruskal-Wallis	0.0292
163	SQDG(34:4)	value	15	12.50	2	0.0019	Kruskal-Wallis	0.0292
148	PI(35:2)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0319
154	SQDG(30:0)	value	15	11.58	2	0.0031	Kruskal-Wallis	0.0319
55	PC(32:0)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0336
70	PC(34:7)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0336
85	PC(36:9)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0336
97	PC(38:9)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0336
130	PG(32:1)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0336
135	PG(34:3)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0336
136	PG(34:4)	value	15	11.18	2	0.0037	Kruskal-Wallis	0.0336
142	PG(36:5)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0336
156	SQDG(32:1)	value	15	11.18	2	0.0037	Kruskal-Wallis	0.0336
157	SQDG(32:2)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0336
158	SQDG(32:3)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0336
159	SQDG(34:0)	value	15	10.82	2	0.0045	Kruskal-Wallis	0.0336
162	SQDG(34:3)	value	15	10.50	2	0.0053	Kruskal-Wallis	0.0336
165	SQDG(36:3)	value	15	10.22	2	0.0060	Kruskal-Wallis	0.0336

83	PC(36:7)	value	15	9.68	2	0.0079	Kruskal-Wallis	0.0400
129	PG(32:0)	value	15	9.62	2	0.0082	Kruskal-Wallis	0.0400
133	PG(34:1)	value	15	9.62	2	0.0082	Kruskal-Wallis	0.0400
164	SQDG(34:5)	value	15	9.78	2	0.0075	Kruskal-Wallis	0.0400
46	MGDG(36:3)	value	15	9.36	2	0.0093	Kruskal-Wallis	0.0408
139	PG(36:2)	value	15	9.38	2	0.0092	Kruskal-Wallis	0.0408
166	SQDG(36:4)	value	15	9.42	2	0.0090	Kruskal-Wallis	0.0408
167	SQDG(36:5)	value	15	9.50	2	0.0087	Kruskal-Wallis	0.0408
141	PG(36:4)	value	15	9.26	2	0.0098	Kruskal-Wallis	0.0418
42	MGDG(34:6)	value	15	8.96	2	0.0113	Kruskal-Wallis	0.0472
35	MGDG(32:5)	value	15	8.88	2	0.0118	Kruskal-Wallis	0.0481
20	DGDG(36:6)	value	15	8.54	2	0.0140	Kruskal-Wallis	0.0531
52	MGMG(16:3)	value	15	8.54	2	0.0140	Kruskal-Wallis	0.0531
140	PG(36:3)	value	15	8.54	2	0.0140	Kruskal-Wallis	0.0531
1	Cer(d35:0)	value	15	8.34	2	0.0155	Kruskal-Wallis	0.0575
43	MGDG(34:7)	value	15	8.24	2	0.0162	Kruskal-Wallis	0.0576
134	PG(34:2)	value	15	8.24	2	0.0162	Kruskal-Wallis	0.0576
71	PC(34:8)	value	15	8.18	2	0.0167	Kruskal-Wallis	0.0581
60	PC(32:5)	value	15	7.28	2	0.0263	Kruskal-Wallis	0.0896
51	MGMG(16:2)	value	15	6.86	2	0.0324	Kruskal-Wallis	0.1082
10	DGDG(34:4)	value	15	6.62	2	0.0365	Kruskal-Wallis	0.1150
93	PC(38:5)	value	15	6.66	2	0.0358	Kruskal-Wallis	0.1150
132	PG(34:0)	value	15	6.62	2	0.0365	Kruskal-Wallis	0.1150
137	PG(36:0)	value	15	6.54	2	0.0380	Kruskal-Wallis	0.1175
105	PE(32:6)	value	15	6.48	2	0.0392	Kruskal-Wallis	0.1190
59	PC(32:4)	value	15	6.32	2	0.0424	Kruskal-Wallis	0.1242
84	PC(36:8)	value	15	6.32	2	0.0424	Kruskal-Wallis	0.1242
92	PC(38:4)	value	15	6.18	2	0.0455	Kruskal-Wallis	0.1310
33	MGDG(32:3)	value	15	6.02	2	0.0493	Kruskal-Wallis	0.1372
128	PE(38:9)	value	15	6.02	2	0.0493	Kruskal-Wallis	0.1372
95	PC(38:7)	value	15	5.78	2	0.0556	Kruskal-Wallis	0.1522
3	DGDG(32:0)	value	15	5.54	2	0.0627	Kruskal-Wallis	0.1614
5	DGDG(32:2)	value	15	5.54	2	0.0627	Kruskal-Wallis	0.1614
8	DGDG(34:2)	value	15	5.46	2	0.0652	Kruskal-Wallis	0.1614
15	DGDG(35:3)	value	15	5.36	2	0.0686	Kruskal-Wallis	0.1614
24	LPC(16:2)	value	15	5.36	2	0.0686	Kruskal-Wallis	0.1614
27	LPC(18:3)	value	15	5.36	2	0.0686	Kruskal-Wallis	0.1614
40	MGDG(34:4)	value	15	5.42	2	0.0665	Kruskal-Wallis	0.1614
96	PC(38:8)	value	15	5.36	2	0.0686	Kruskal-Wallis	0.1614
131	PG(33:0)	value	15	5.58	2	0.0614	Kruskal-Wallis	0.1614
138	PG(36:1)	value	15	5.46	2	0.0652	Kruskal-Wallis	0.1614
4	DGDG(32:1)	value	15	5.18	2	0.0750	Kruskal-Wallis	0.1716
88	PC(37:4)	value	15	5.18	2	0.0750	Kruskal-Wallis	0.1716
61	PC(32:6)	value	15	5.04	2	0.0805	Kruskal-Wallis	0.1769
99	PE(30:3)	value	15	5.04	2	0.0805	Kruskal-Wallis	0.1769
118	PE(36:4-OH)	value	15	5.04	2	0.0805	Kruskal-Wallis	0.1769
69	PC(34:6)	value	15	4.88	2	0.0872	Kruskal-Wallis	0.1891
34	MGDG(32:4)	value	15	4.82	2	0.0898	Kruskal-Wallis	0.1923
57	PC(32:2)	value	15	4.56	2	0.1020	Kruskal-Wallis	0.2156
11	DGDG(34:5)	value	15	4.46	2	0.1080	Kruskal-Wallis	0.2255
9	DGDG(34:3)	value	15	4.34	2	0.1140	Kruskal-Wallis	0.2294
23	LPC(16:0)	value	15	4.34	2	0.1140	Kruskal-Wallis	0.2294
121	PE(36:6)	value	15	4.34	2	0.1140	Kruskal-Wallis	0.2294

63	PC(33:3)	value	15	4.22	2	0.1210	Kruskal-Wallis	0.2406
81	PC(36:5)	value	15	4.16	2	0.1250	Kruskal-Wallis	0.2456
79	PC(36:4-OH)	value	15	3.92	2	0.1410	Kruskal-Wallis	0.2707
153	PI_Cer(t34:0-OH)	value	15	3.92	2	0.1410	Kruskal-Wallis	0.2707
31	LPE(20:5)	value	15	3.86	2	0.1450	Kruskal-Wallis	0.2721
37	MGDG(34:1)	value	15	3.86	2	0.1450	Kruskal-Wallis	0.2721
45	MGDG(36:2)	value	15	3.78	2	0.1510	Kruskal-Wallis	0.2771
124	PE(36:9)	value	15	3.78	2	0.1510	Kruskal-Wallis	0.2771
21	DGDG(36:7)	value	15	3.62	2	0.1640	Kruskal-Wallis	0.2945
110	PE(34:4)	value	15	3.62	2	0.1640	Kruskal-Wallis	0.2945
13	DGDG(35:1)	value	15	3.44	2	0.1790	Kruskal-Wallis	0.3053
14	DGDG(35:2)	value	15	3.44	2	0.1790	Kruskal-Wallis	0.3053
29	LPE(18:1)	value	15	3.50	2	0.1740	Kruskal-Wallis	0.3053
82	PC(36:6)	value	15	3.50	2	0.1740	Kruskal-Wallis	0.3053
94	PC(38:6)	value	15	3.42	2	0.1810	Kruskal-Wallis	0.3053
117	PE(36:3)	value	15	3.42	2	0.1810	Kruskal-Wallis	0.3053
56	PC(32:1)	value	15	3.26	2	0.1960	Kruskal-Wallis	0.3209
98	PE(30:2)	value	15	3.26	2	0.1960	Kruskal-Wallis	0.3209
126	PE(38:7)	value	15	3.26	2	0.1960	Kruskal-Wallis	0.3209
26	LPC(18:2)	value	15	3.14	2	0.2080	Kruskal-Wallis	0.3372
116	PE(36:2)	value	15	3.12	2	0.2100	Kruskal-Wallis	0.3372
22	DGMG(16:0)	value	15	2.94	2	0.2300	Kruskal-Wallis	0.3658
6	DGDG(32:3)	value	15	2.78	2	0.2490	Kruskal-Wallis	0.3886
49	MGDG(36:6)	value	15	2.78	2	0.2490	Kruskal-Wallis	0.3886
39	MGDG(34:3)	value	15	2.54	2	0.2810	Kruskal-Wallis	0.4345
25	LPC(18:1)	value	15	2.48	2	0.2890	Kruskal-Wallis	0.4348
68	PC(34:5)	value	15	2.48	2	0.2890	Kruskal-Wallis	0.4348
89	PC(37:5)	value	15	2.48	2	0.2890	Kruskal-Wallis	0.4348
7	DGDG(34:1)	value	15	2.42	2	0.2980	Kruskal-Wallis	0.4365
36	MGDG(34:0)	value	15	2.42	2	0.2980	Kruskal-Wallis	0.4365
67	PC(34:4)	value	15	2.42	2	0.2980	Kruskal-Wallis	0.4365
19	DGDG(36:5)	value	15	2.34	2	0.3100	Kruskal-Wallis	0.4463
41	MGDG(34:5)	value	15	2.34	2	0.3100	Kruskal-Wallis	0.4463
32	MGDG(32:2)	value	15	2.24	2	0.3260	Kruskal-Wallis	0.4631
77	PC(36:2)	value	15	2.22	2	0.3300	Kruskal-Wallis	0.4631
127	PE(38:8)	value	15	2.22	2	0.3300	Kruskal-Wallis	0.4631
122	PE(36:7)	value	15	2.06	2	0.3570	Kruskal-Wallis	0.4968
17	DGDG(36:3)	value	15	2.00	2	0.3680	Kruskal-Wallis	0.5079
16	DGDG(36:2)	value	15	1.94	2	0.3790	Kruskal-Wallis	0.5146
53	MGMG(18:2)	value	15	1.94	2	0.3790	Kruskal-Wallis	0.5146
50	MGMG(16:0)	value	15	1.82	2	0.4030	Kruskal-Wallis	0.5258
64	PC(34:1)	value	15	1.86	2	0.3950	Kruskal-Wallis	0.5258
76	PC(36:1)	value	15	1.86	2	0.3950	Kruskal-Wallis	0.5258
103	PE(32:4)	value	15	1.82	2	0.4030	Kruskal-Wallis	0.5258
125	PE(37:5)	value	15	1.82	2	0.4030	Kruskal-Wallis	0.5258
90	PC(38:2)	value	15	1.68	2	0.4320	Kruskal-Wallis	0.5550
107	PE(34:1)	value	15	1.68	2	0.4320	Kruskal-Wallis	0.5550
12	DGDG(34:6)	value	15	1.62	2	0.4450	Kruskal-Wallis	0.5673
18	DGDG(36:4)	value	15	1.58	2	0.4540	Kruskal-Wallis	0.5744
65	PC(34:2)	value	15	1.50	2	0.4720	Kruskal-Wallis	0.5839
100	PE(32:1)	value	15	1.50	2	0.4720	Kruskal-Wallis	0.5839
104	PE(32:5)	value	15	1.52	2	0.4680	Kruskal-Wallis	0.5839
111	PE(34:5)	value	15	1.46	2	0.4820	Kruskal-Wallis	0.5919

30	LPE(18:2)	value	15	1.34	2	0.5120	Kruskal-Wallis	0.6241
66	PC(34:3)	value	15	1.26	2	0.5330	Kruskal-Wallis	0.6313
75	PC(35:4)	value	15	1.28	2	0.5270	Kruskal-Wallis	0.6313
80	PC(36:4)	value	15	1.26	2	0.5330	Kruskal-Wallis	0.6313
108	PE(34:2)	value	15	1.26	2	0.5330	Kruskal-Wallis	0.6313
91	PC(38:3)	value	15	1.14	2	0.5660	Kruskal-Wallis	0.6610
102	PE(32:3)	value	15	1.14	2	0.5660	Kruskal-Wallis	0.6610
28	LPE(16:0)	value	15	0.98	2	0.6130	Kruskal-Wallis	0.6938
44	MGDG(35:1)	value	15	0.98	2	0.6130	Kruskal-Wallis	0.6938
74	PC(35:3)	value	15	0.96	2	0.6190	Kruskal-Wallis	0.6938
109	PE(34:3)	value	15	0.98	2	0.6130	Kruskal-Wallis	0.6938
114	PE(35:3)	value	15	0.98	2	0.6130	Kruskal-Wallis	0.6938
120	PE(36:5)	value	15	0.96	2	0.6190	Kruskal-Wallis	0.6938
62	PC(33:2)	value	15	0.86	2	0.6510	Kruskal-Wallis	0.7248
38	MGDG(34:2)	value	15	0.74	2	0.6910	Kruskal-Wallis	0.7520
54	PC(30:3)	value	15	0.74	2	0.6910	Kruskal-Wallis	0.7520
58	PC(32:3)	value	15	0.74	2	0.6910	Kruskal-Wallis	0.7520
72	PC(35:1)	value	15	0.72	2	0.6980	Kruskal-Wallis	0.7520
78	PC(36:3)	value	15	0.72	2	0.6980	Kruskal-Wallis	0.7520
73	PC(35:2)	value	15	0.62	2	0.7330	Kruskal-Wallis	0.7847
47	MGDG(36:4)	value	15	0.54	2	0.7630	Kruskal-Wallis	0.8116
87	PC(37:3)	value	15	0.42	2	0.8110	Kruskal-Wallis	0.8465
115	PE(35:4)	value	15	0.42	2	0.8110	Kruskal-Wallis	0.8465
119	PE(36:4)	value	15	0.42	2	0.8110	Kruskal-Wallis	0.8465
48	MGDG(36:5)	value	15	0.38	2	0.8270	Kruskal-Wallis	0.8525
112	PE(34:6)	value	15	0.38	2	0.8270	Kruskal-Wallis	0.8525
86	PC(37:2)	value	15	0.26	2	0.8780	Kruskal-Wallis	0.8941
113	PE(35:2)	value	15	0.26	2	0.8780	Kruskal-Wallis	0.8941
106	PE(33:3)	value	15	0.08	2	0.9610	Kruskal-Wallis	0.9726
101	PE(32:2)	value	15	0.06	2	0.9700	Kruskal-Wallis	0.9758
123	PE(36:8)	value	15	0.02	2	0.9900	Kruskal-Wallis	0.9900

Abbreviations: C-Hetero, *C. vulgaris* cultivated under heterotrophic conditions; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; Cer, Ceramide; PI_Cer, inositolphosphoceramide; TG, triglyceride.

Table S3.6. Results of Dunn's multiple comparison post-hoc test (FDR adjusted) using log normalized peak areas of significant molecular ions (*q* values < 0.05) of polar lipids identified in DCM, EtOH and UAE extracts from C-Hetero.

	variable	.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
1	DGDG(28:0)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0047	**
2	DGDG(28:0)	value	DCM	UAE	5	5	-1.77	0.0771	0.1103	ns
3	DGDG(28:0)	value	EtOH	UAE	5	5	1.77	0.0771	0.1103	ns
4	MGDG(32:5)	value	DCM	EtOH	5	5	-1.27	0.2031	0.2425	ns
5	MGDG(32:5)	value	DCM	UAE	5	5	-2.97	0.0030	0.0102	*
6	MGDG(32:5)	value	EtOH	UAE	5	5	-1.70	0.0897	0.1268	ns
7	MGDG(34:6)	value	DCM	EtOH	5	5	-2.26	0.0237	0.0619	ns
8	MGDG(34:6)	value	DCM	UAE	5	5	-2.83	0.0047	0.0148	*

9	MGDG(34:6)	value	EtOH	UAE	5	5	-0.57	0.5716	0.5908	ns
10	MGDG(36:3)	value	DCM	EtOH	5	5	-2.12	0.0339	0.0719	ns
11	MGDG(36:3)	value	DCM	UAE	5	5	-2.97	0.0030	0.0102	*
12	MGDG(36:3)	value	EtOH	UAE	5	5	-0.85	0.3961	0.4165	ns
13	PC(32:0)	value	DCM	EtOH	5	5	-0.92	0.3580	0.3796	ns
14	PC(32:0)	value	DCM	UAE	5	5	-3.11	0.0019	0.0074	**
15	PC(32:0)	value	EtOH	UAE	5	5	-2.19	0.0284	0.0671	ns
16	PC(34:7)	value	DCM	EtOH	5	5	-0.92	0.3580	0.3796	ns
17	PC(34:7)	value	DCM	UAE	5	5	-3.11	0.0019	0.0074	**
18	PC(34:7)	value	EtOH	UAE	5	5	-2.19	0.0284	0.0671	ns
19	PC(36:7)	value	DCM	EtOH	5	5	-1.56	0.1198	0.1519	ns
20	PC(36:7)	value	DCM	UAE	5	5	-3.11	0.0019	0.0074	**
21	PC(36:7)	value	EtOH	UAE	5	5	-1.56	0.1198	0.1519	ns
22	PC(36:9)	value	DCM	EtOH	5	5	-2.19	0.0284	0.0671	ns
23	PC(36:9)	value	DCM	UAE	5	5	-3.11	0.0019	0.0074	**
24	PC(36:9)	value	EtOH	UAE	5	5	-0.92	0.3580	0.3796	ns
25	PC(38:9)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0072	**
26	PC(38:9)	value	DCM	UAE	5	5	-2.12	0.0339	0.0719	ns
27	PC(38:9)	value	EtOH	UAE	5	5	1.06	0.2888	0.3230	ns
28	PG(32:0)	value	DCM	EtOH	5	5	-2.90	0.0037	0.0124	*
29	PG(32:0)	value	DCM	UAE	5	5	-2.40	0.0162	0.0443	*
30	PG(32:0)	value	EtOH	UAE	5	5	0.49	0.6206	0.6361	ns
31	PG(32:1)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0072	**
32	PG(32:1)	value	DCM	UAE	5	5	-1.06	0.2888	0.3230	ns
33	PG(32:1)	value	EtOH	UAE	5	5	2.12	0.0339	0.0719	ns
34	PG(34:1)	value	DCM	EtOH	5	5	-3.04	0.0024	0.0088	**
35	PG(34:1)	value	DCM	UAE	5	5	-0.99	0.3222	0.3570	ns
36	PG(34:1)	value	EtOH	UAE	5	5	2.05	0.0403	0.0813	ns
37	PG(34:3)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0070	**
38	PG(34:3)	value	DCM	UAE	5	5	-2.05	0.0403	0.0813	ns
39	PG(34:3)	value	EtOH	UAE	5	5	1.20	0.2293	0.2686	ns
40	PG(34:4)	value	DCM	EtOH	5	5	-3.32	0.0009	0.0061	**
41	PG(34:4)	value	DCM	UAE	5	5	-1.98	0.0477	0.0932	ns
42	PG(34:4)	value	EtOH	UAE	5	5	1.34	0.1791	0.2160	ns
43	PG(36:2)	value	DCM	EtOH	5	5	-2.69	0.0072	0.0216	*
44	PG(36:2)	value	DCM	UAE	5	5	-2.62	0.0089	0.0260	*
45	PG(36:2)	value	EtOH	UAE	5	5	0.07	0.9436	0.9436	ns
46	PG(36:4)	value	DCM	EtOH	5	5	-3.04	0.0024	0.0088	**
47	PG(36:4)	value	DCM	UAE	5	5	-1.63	0.1039	0.1345	ns
48	PG(36:4)	value	EtOH	UAE	5	5	1.41	0.1573	0.1935	ns
49	PG(36:5)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0072	**
50	PG(36:5)	value	DCM	UAE	5	5	-1.06	0.2888	0.3230	ns
51	PG(36:5)	value	EtOH	UAE	5	5	2.12	0.0339	0.0719	ns
52	PG(38:5)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0047	**
53	PG(38:5)	value	DCM	UAE	5	5	-1.84	0.0660	0.1103	ns
54	PG(38:5)	value	EtOH	UAE	5	5	1.63	0.1039	0.1345	ns
55	PI(32:2)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0047	**
56	PI(32:2)	value	DCM	UAE	5	5	-1.77	0.0771	0.1103	ns
57	PI(32:2)	value	EtOH	UAE	5	5	1.77	0.0771	0.1103	ns
58	PI(34:1)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0047	**
59	PI(34:1)	value	DCM	UAE	5	5	-1.77	0.0771	0.1103	ns
60	PI(34:1)	value	EtOH	UAE	5	5	1.77	0.0771	0.1103	ns
61	PI(34:2)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0047	**

62	PI(34:2)	value	DCM	UAE	5	5	-1.63	0.1039	0.1345	ns
63	PI(34:2)	value	EtOH	UAE	5	5	1.84	0.0660	0.1103	ns
64	PI(34:3)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0047	**
65	PI(34:3)	value	DCM	UAE	5	5	-1.63	0.1039	0.1345	ns
66	PI(34:3)	value	EtOH	UAE	5	5	1.84	0.0660	0.1103	ns
67	PI(35:2)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0053	**
68	PI(35:2)	value	DCM	UAE	5	5	-1.48	0.1376	0.1709	ns
69	PI(35:2)	value	EtOH	UAE	5	5	1.91	0.0562	0.1064	ns
70	PI(36:2)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0047	**
71	PI(36:2)	value	DCM	UAE	5	5	-1.63	0.1039	0.1345	ns
72	PI(36:2)	value	EtOH	UAE	5	5	1.84	0.0660	0.1103	ns
73	PI(36:3)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0047	**
74	PI(36:3)	value	DCM	UAE	5	5	-1.77	0.0771	0.1103	ns
75	PI(36:3)	value	EtOH	UAE	5	5	1.77	0.0771	0.1103	ns
76	PI(36:4)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0047	**
77	PI(36:4)	value	DCM	UAE	5	5	-1.77	0.0771	0.1103	ns
78	PI(36:4)	value	EtOH	UAE	5	5	1.77	0.0771	0.1103	ns
79	PI(36:5)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0047	**
80	PI(36:5)	value	DCM	UAE	5	5	-1.77	0.0771	0.1103	ns
81	PI(36:5)	value	EtOH	UAE	5	5	1.77	0.0771	0.1103	ns
82	SQDG(30:0)	value	DCM	EtOH	5	5	-3.39	0.0007	0.0053	**
83	SQDG(30:0)	value	DCM	UAE	5	5	-1.48	0.1376	0.1709	ns
84	SQDG(30:0)	value	EtOH	UAE	5	5	1.91	0.0562	0.1064	ns
85	SQDG(32:0)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0047	**
86	SQDG(32:0)	value	DCM	UAE	5	5	-1.63	0.1039	0.1345	ns
87	SQDG(32:0)	value	EtOH	UAE	5	5	1.84	0.0660	0.1103	ns
88	SQDG(32:1)	value	DCM	EtOH	5	5	-3.32	0.0009	0.0061	**
89	SQDG(32:1)	value	DCM	UAE	5	5	-1.34	0.1791	0.2160	ns
90	SQDG(32:1)	value	EtOH	UAE	5	5	1.98	0.0477	0.0932	ns
91	SQDG(32:2)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0072	**
92	SQDG(32:2)	value	DCM	UAE	5	5	-1.06	0.2888	0.3230	ns
93	SQDG(32:2)	value	EtOH	UAE	5	5	2.12	0.0339	0.0719	ns
94	SQDG(32:3)	value	DCM	EtOH	5	5	-3.11	0.0019	0.0074	**
95	SQDG(32:3)	value	DCM	UAE	5	5	-0.92	0.3580	0.3796	ns
96	SQDG(32:3)	value	EtOH	UAE	5	5	2.19	0.0284	0.0671	ns
97	SQDG(34:0)	value	DCM	EtOH	5	5	-3.25	0.0011	0.0070	**
98	SQDG(34:0)	value	DCM	UAE	5	5	-1.20	0.2293	0.2686	ns
99	SQDG(34:0)	value	EtOH	UAE	5	5	2.05	0.0403	0.0813	ns
100	SQDG(34:1)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0047	**
101	SQDG(34:1)	value	DCM	UAE	5	5	-1.63	0.1039	0.1345	ns
102	SQDG(34:1)	value	EtOH	UAE	5	5	1.84	0.0660	0.1103	ns
103	SQDG(34:2)	value	DCM	EtOH	5	5	-3.46	0.0005	0.0047	**
104	SQDG(34:2)	value	DCM	UAE	5	5	-1.84	0.0660	0.1103	ns
105	SQDG(34:2)	value	EtOH	UAE	5	5	1.63	0.1039	0.1345	ns
106	SQDG(34:3)	value	DCM	EtOH	5	5	-3.18	0.0015	0.0072	**
107	SQDG(34:3)	value	DCM	UAE	5	5	-1.06	0.2888	0.3230	ns
108	SQDG(34:3)	value	EtOH	UAE	5	5	2.12	0.0339	0.0719	ns
109	SQDG(34:4)	value	DCM	EtOH	5	5	-3.54	0.0004	0.0047	**
110	SQDG(34:4)	value	DCM	UAE	5	5	-1.77	0.0771	0.1103	ns
111	SQDG(34:4)	value	EtOH	UAE	5	5	1.77	0.0771	0.1103	ns
112	SQDG(34:5)	value	DCM	EtOH	5	5	-2.97	0.0030	0.0102	*
113	SQDG(34:5)	value	DCM	UAE	5	5	-0.64	0.5245	0.5467	ns
114	SQDG(34:5)	value	EtOH	UAE	5	5	2.33	0.0196	0.0525	ns

115	SQDG(36:3)	value	DCM	EtOH	5	5	-3.11	0.0019	0.0074	**
116	SQDG(36:3)	value	DCM	UAE	5	5	-0.92	0.3580	0.3796	ns
117	SQDG(36:3)	value	EtOH	UAE	5	5	2.19	0.0284	0.0671	ns
118	SQDG(36:4)	value	DCM	EtOH	5	5	-2.76	0.0058	0.0179	*
119	SQDG(36:4)	value	DCM	UAE	5	5	-0.21	0.8320	0.8388	ns
120	SQDG(36:4)	value	EtOH	UAE	5	5	2.55	0.0109	0.0312	*
121	SQDG(36:5)	value	DCM	EtOH	5	5	-2.83	0.0047	0.0148	*
122	SQDG(36:5)	value	DCM	UAE	5	5	-0.35	0.7237	0.7356	ns
123	SQDG(36:5)	value	EtOH	UAE	5	5	2.47	0.0133	0.0373	*

Abbreviations: C-Hetero, *C. vulgaris* cultivated under heterotrophic conditions; DCM, dichloromethane:methanol (2:1, v/v) ; EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; Cer, ceramide; PI_Cer, inositolphosphoceramide; TG, triglyceride.

Table S3.7. List of polar lipid species displaying higher amounts in UAE extracts, compared with EtOH extracts from C-Auto. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains. The identification of the fatty acyl chains of each lipid species was assigned based on previous publication [10]. Bold lipid species correspond to the most abundant species in each class detected.

Lipid species (C:N) of the C-Auto	Fatty acyl chains observed in MS/MS of the C-Auto
PG(36:2)	(18:1/18:1) and (18:0/18:2)
PE(36:6)	(18:3/18:3)
PE(36:4)	(18:2/18:2) and (18:1/18:3)
PI(34:3)	(16:0/18:3), (16:1/18:2) and (18:0/16:3)
LPC(16:0)	(16:0)
LPC(18:3)	(18:3)
LPC(18:2)	(18:2)
MGMG(16:3)	–
MGMG(18:3)	**(18:3)**
SQDG(32:0)	**(16:0/16:0)**
SQDG(34:3)	**(18:3/16:0)**
SQDG(34:2)	(18:2/16:0)
SQDG(32:1)	(16:1/16:0)
SQDG(36:6)	(18:3/18:3)
DGDG(34:6)	**(18:3/16:3)**
DGDG(34:3)	(18:3/16:0), (18:2/16:1) and (18:1/16:2)
DGDG(36:5)	(18:3/18:2)

Table S3.8. List of polar lipid species displaying higher amounts in UAE extracts, compared with EtOH extracts from C-Hetero. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains. The identification of the fatty acyl chains of each lipid species was assigned based on previous publication [10]. Bold lipid species correspond to the most abundant species in each class detected.

Lipid species (C:N) of the C-Hetero	**Fatty acyl chains observed in MS/MS of the C-Hetero**
PG(32:0)	(16:0/16:0)
PG(34:3)	(16:0/18:3)
PG(34:1)	**(17:0/17:1) and (16:0/18:1)**
PI(34:3)	(16:0/18:3), (16:1/18:2), (18:0/16:3) and (18:1/16:2)
PI(32:2)	(14:1/18:1); (16:0/16:2) and (14:0/18:2)
PI(34:2)	**(16:0/18:2)**
PI(34:1)	_
PI(35:2)	(17:0/18:2)
PI(36:3)	(18:1/18:2)
SQDG(32:0)	**(16:0/16:0)**
SQDG(34:3)	(18:3/16:0)
SQDG(34:2)	(18:2/16:0)
SQDG(34:1)	_
SQDG(34:0)	_
SQDG(36:4)	(18:2/18:2)

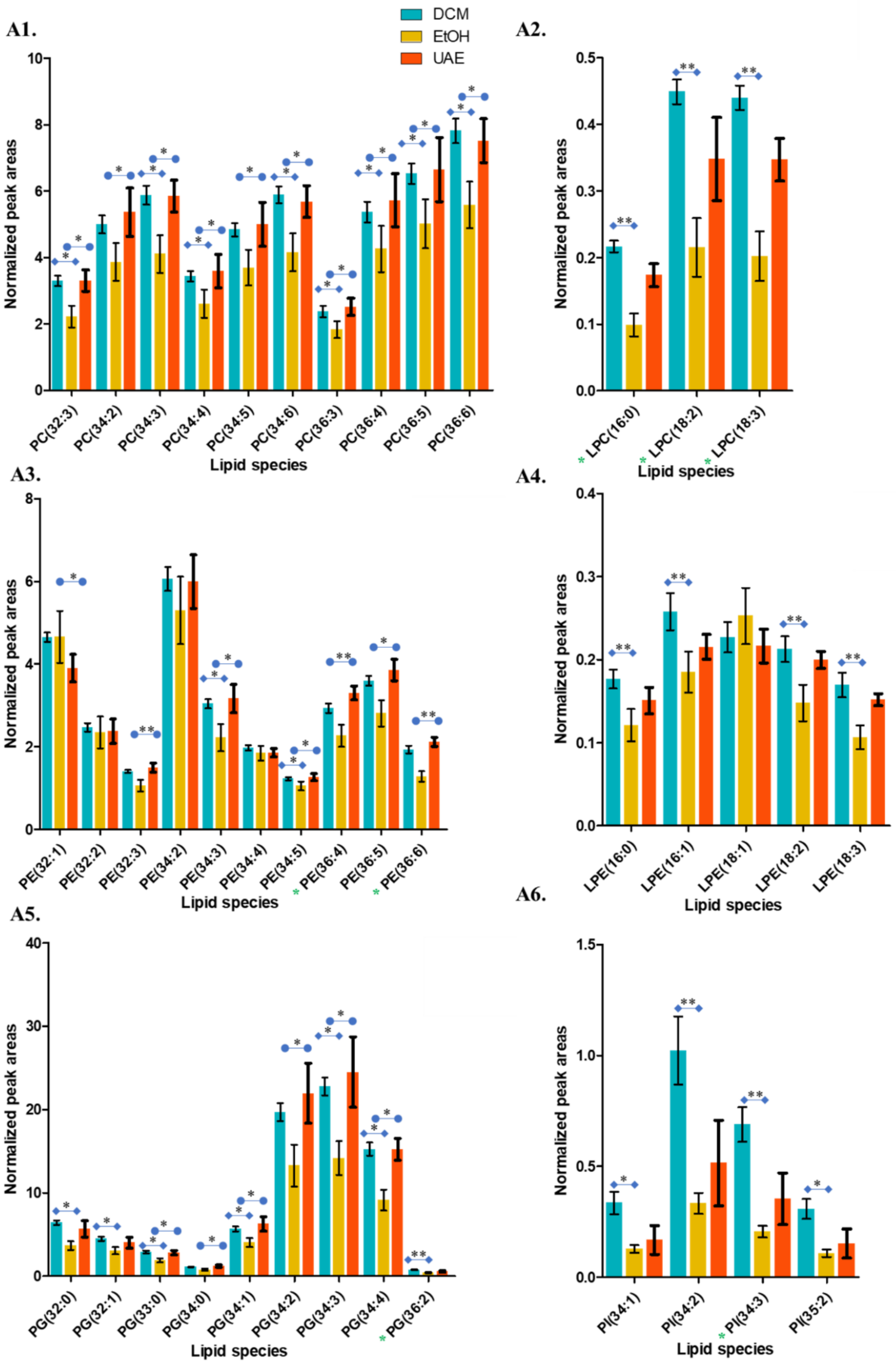


Figure S3.4. Most abundant phospholipid species identified in DCM, EtOH and UAE extracts from C-Auto. (**A1**) phosphatidylcholine (PC); (**A2**) lysophosphatidylcholine (LPC); (**A3**) phosphatidylethanolamine (PE); (**A4**) lysophosphatidylethanolamine (LPE); (**A5**) phosphatidylglycerol (PG); and (**A6**) phosphatidylinositol (PI). The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments. Statistic significant differences (*q*-value<0.05) are annotated with "*". *Lipid species displayed in the top 40 of the most significant lipid species. Abbreviations: C-Auto, *C. vulgaris* cultivated under autotrophic conditions; DCM, dichloromethane:methanol (2:1, v/v) ; EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe.

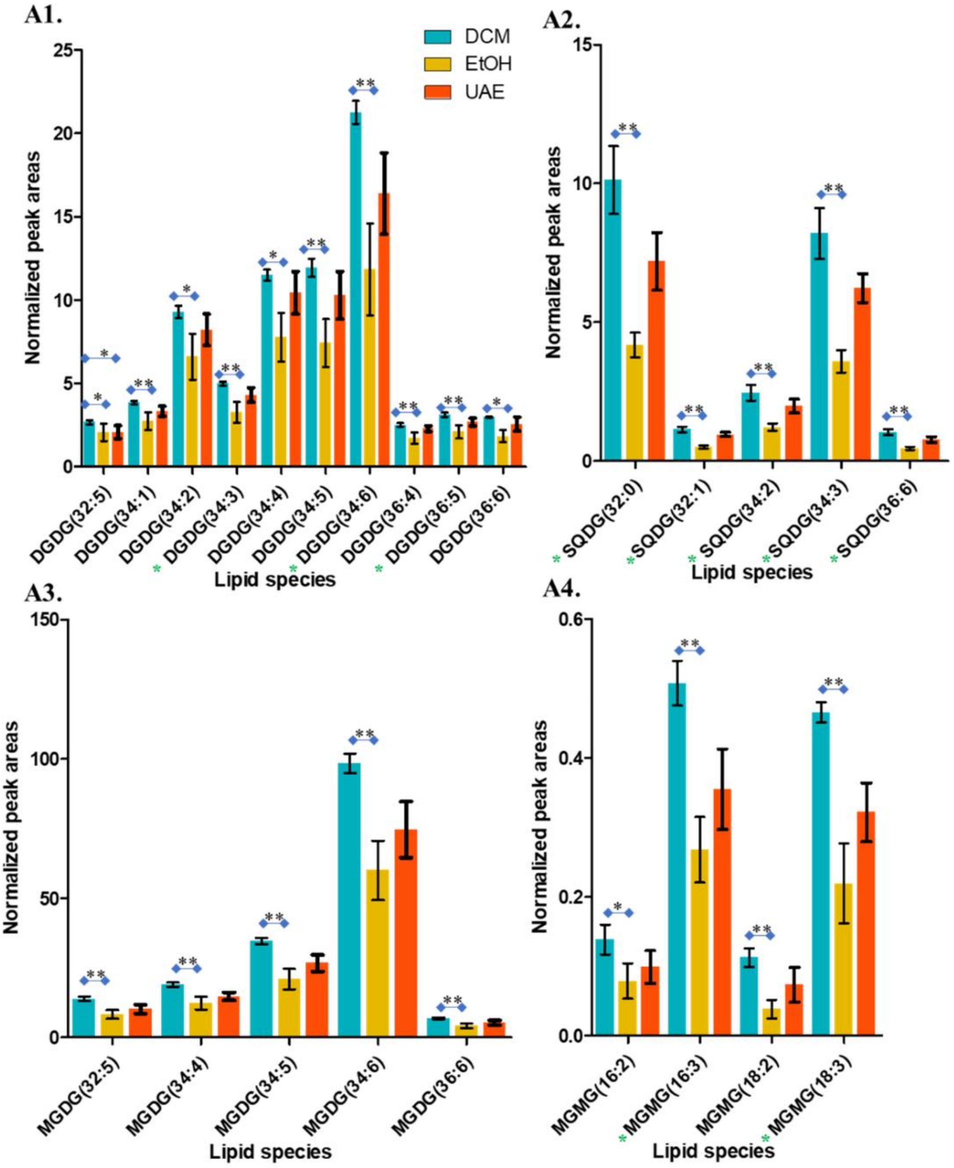


Figure S3.5. Most abundant glycolipid species identified in DCM, EtOH and UAE extracts from C-Auto. (**A1**) digalactosyldiacylglycerol (DGDG), (**A2**) sulfoquinovosyldiacylglycerol (SQDG); (**A3**) monogalactosyldiacylglycerol (MGDG); (**A4**) monogalactosylmonoacylglycerol (MGMG) lipid species. The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments. Statistic significant differences (*q*-value<0.05) are annotated with "*". *Lipid species displayed in the top 40 of the most significant lipid species. Abbreviations: C-Auto, C. vulgaris cultivated under autotrophic conditions; DCM, dichloromethane:methanol (2:1, v/v); EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe.

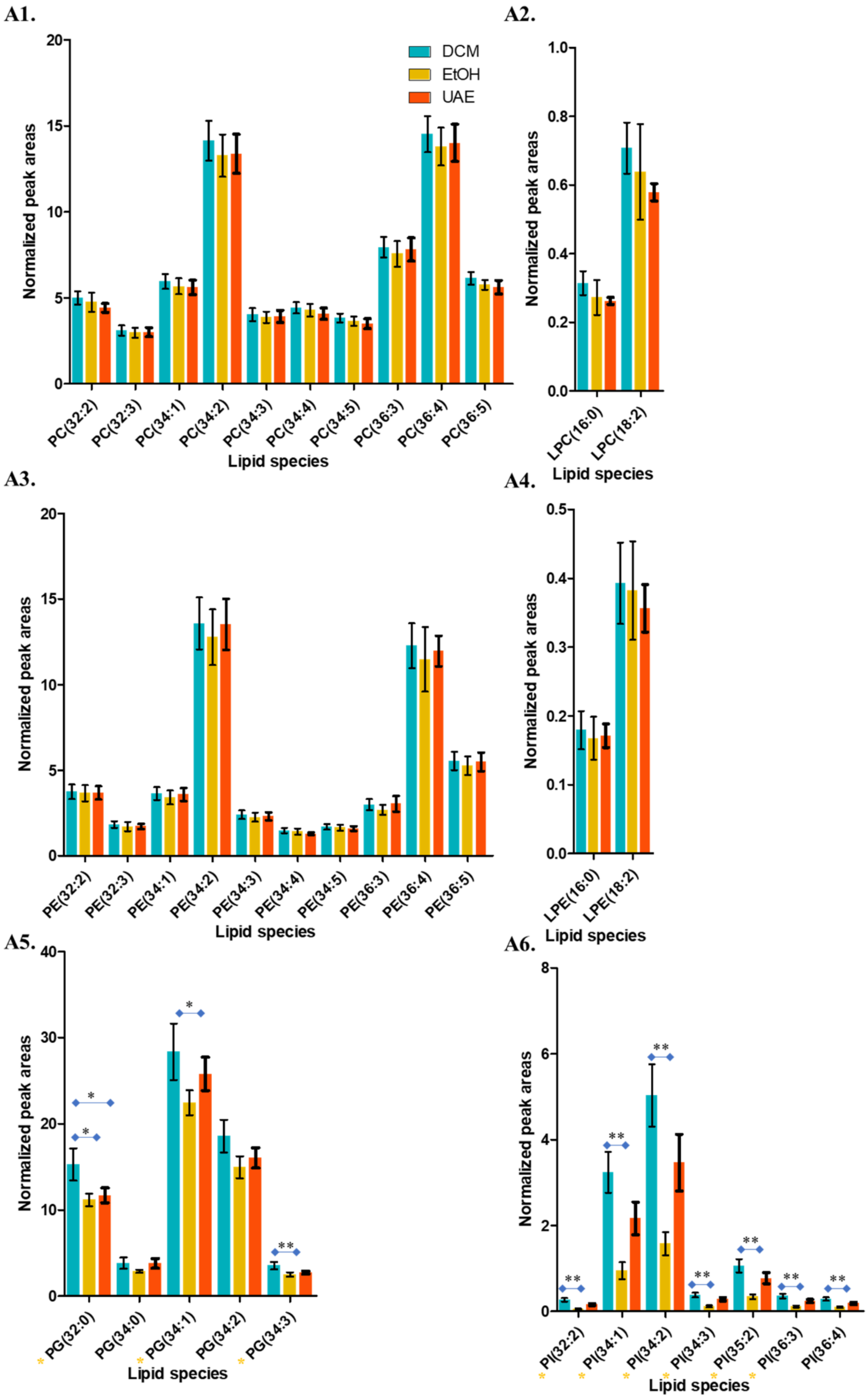

A1.
DCM
EtOH
UAE
Normalized peak areas
0
5
10
15
20
PC(32:2)
PC(32:3)
PC(34:1)
PC(34:2)
PC(34:3)
PC(34:4)
PC(34:5)
PC(36:3)
PC(36:4)
PC(36:5)
Lipid species
A2.
Normalized peak areas
0.0
0.2
0.4
0.6
0.8
1.0
LPC(16:0)
LPC(18:2)
Lipid species
A3.
Normalized peak areas
0
5
10
15
20
PE(32:2)
PE(32:3)
PE(34:1)
PE(34:2)
PE(34:3)
PE(34:4)
PE(34:5)
PE(36:3)
PE(36:4)
PE(36:5)
Lipid species
A4.
Normalized peak areas
0.0
0.1
0.2
0.3
0.4
0.5
LPE(16:0)
LPE(18:2)
Lipid species
A5.
Normalized peak areas
0
10
20
30
40
*
*
*
**
PG(32:0)
PG(34:0)
PG(34:1)
PG(34:2)
PG(34:3)
Lipid species
A6.
Normalized peak areas
0
2
4
6
8
**
**
**
**
**
**
**
PI(32:2)
PI(34:1)
PI(34:2)
PI(34:3)
PI(35:2)
PI(36:3)
PI(36:4)
Lipid species

Figure S3.6. Most abundant phospholipid species identified in DCM, EtOH and UAE extracts from C-Hetero. (**A1**) phosphatidylcholine (PC); (**A2**) lysophosphatidylcholine (LPC); (**A3**) phosphatidylethanolamine (PE); (**A4**) lysophosphatidylethanolamine (LPE); (**A5**) phosphatidylglycerol (PG); and (**A6**) phosphatidylinositol (PI). The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments. Statistic significant differences (*q*-value<0.05) are annotated with "*". *Lipid species displayed in the top 40 of the most significant lipid species. Abbreviations: C-Hetero, *C. vulgaris* cultivated under heterotrophic conditions; DCM, dichloromethane:methanol (2:1, *v/v*); EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe.

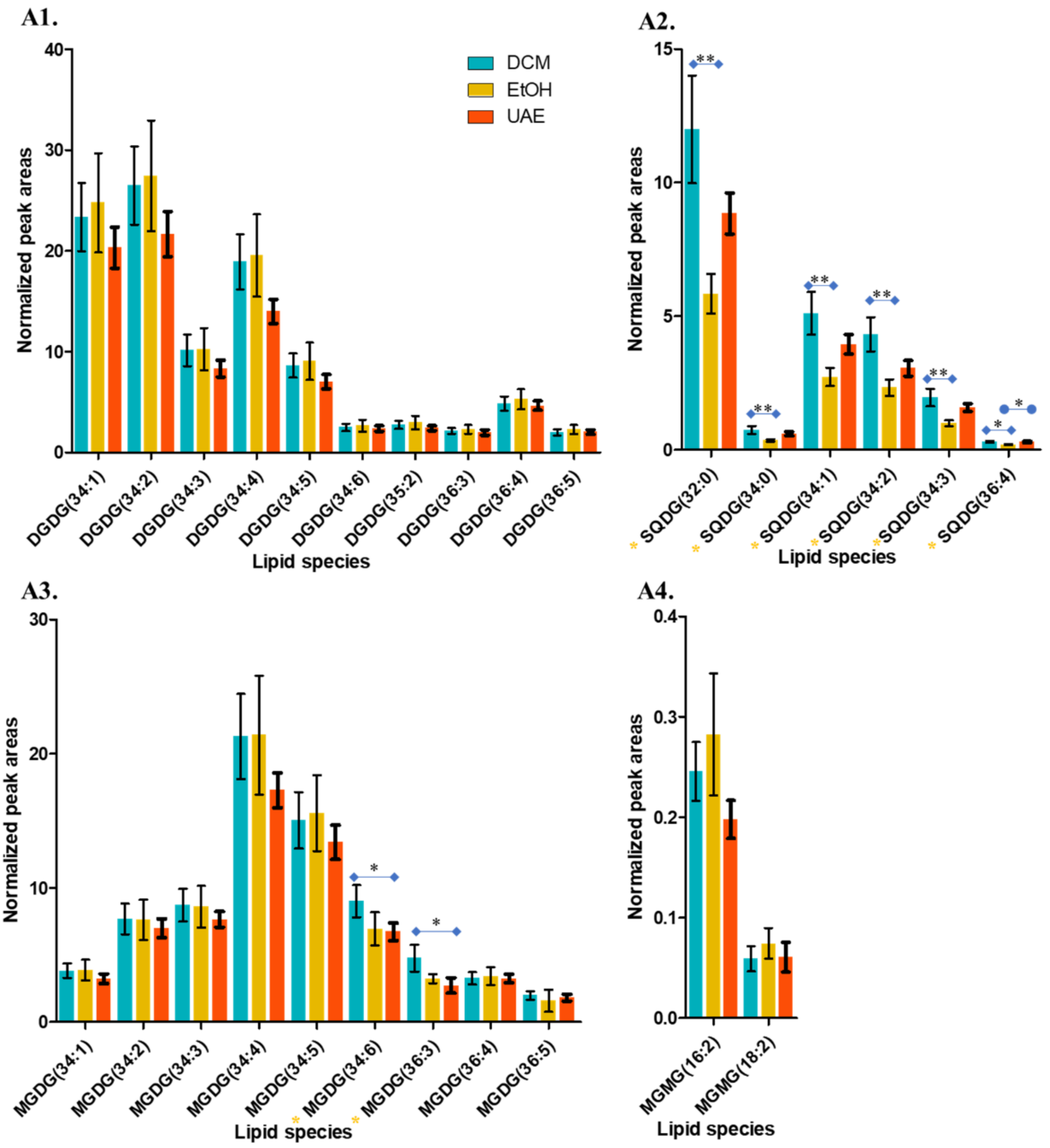


Figure S3.7. Most abundant glycolipid species identified in DCM, EtOH and UAE extracts from C-Hetero. (**A1**) digalactosyldiacylglycerol (DGDG), (**A2**) sulfoquinovosyldiacylglycerol (SQDG); (**A3**) monogalactosyldiacylglycerol (MGDG); (**A4**) monogalactosylmonoacylglycerol (MGMG) lipid species. The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments. Statistic significant differences (*q*-value<0.05) are annotated with “*”. *Lipid species displayed in the top 40 of the most significant lipid species. Abbreviations: C-Hetero, *C. vulgaris* cultivated under heterotrophic conditions; DCM, dichloromethane:methanol (2:1, *v/v*); EtOH, ethanol; UAE, ethanol extraction assisted by an ultrasound probe.

Supplementary material of Chapter 4

Table S4.1. Lipid content (mg/100 mg biomass DW) of the four *C. vulgaris* strains (C-White, C-Honey, C-Auto (wild-type) and C-Hetero). Data are presented as mean ± standard deviation (SD) (n = 5).

C. vulgaris strains	Total lipid extract (%) (mg/100 mg biomass DW)	Ref
C-White	4.8±0.5	-
C-Honey	5.0±1.2	-
C-Auto	12.5±0.9	[9]
C-Hetero	7.9±0.7	[9]

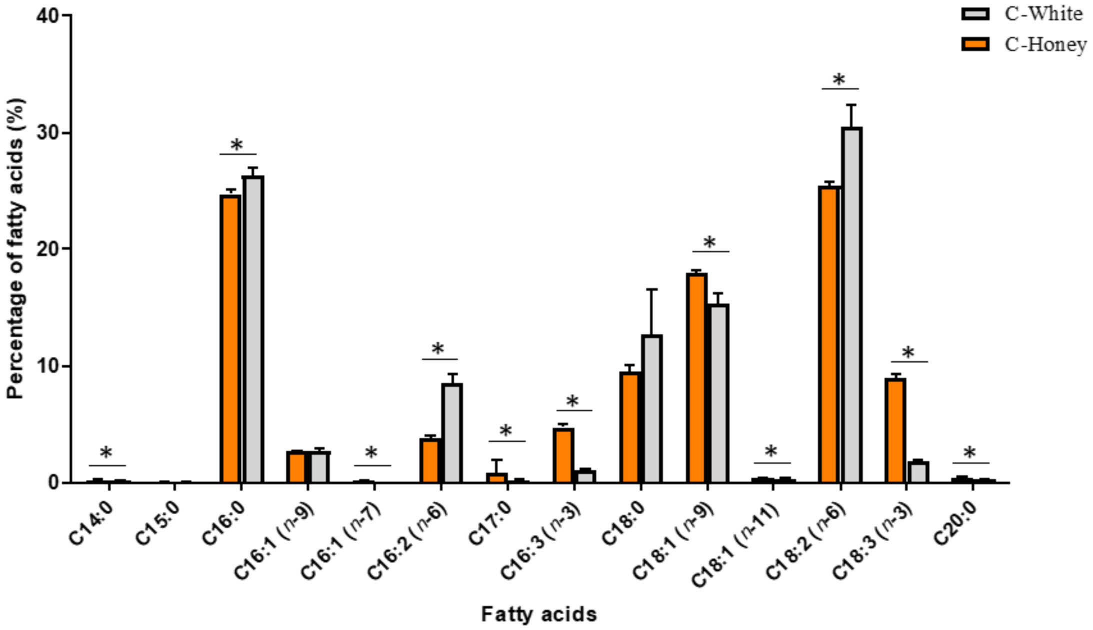


Figure S4.1. Fatty acid profile of C-Honey and C-White *C. vulgaris*. Data are presented as mean ± standard deviation (SD) (n = 5). *Statistically significant differences between *C. vulgaris* mutants (*Wilcoxon rank sum tes*t, $q < 0.05$).

Table S4.2. Results of Wilcoxon rank-sum test (FDR adjusted) for statistically significant differences of fatty acids and lipidic quality indexes between C-Honey and C- White.

Variable	**group1**	**group2**	**n1**	**n2**	**statistic**	**p**	**p.adj**	**p.adj.signif**
C16:0	C-White	C-Honey	5	5	25	0,00794	0,0110278	*
C16.1.*n*.7.	C-White	C-Honey	5	5	0	0,00794	0,0110278	*
C16.2.*n*.6.	C-White	C-Honey	5	5	25	0,00794	0,0110278	*
C17.0	C-White	C-Honey	5	5	0	0,00794	0,0110278	*
C16.3.*n*.3.	C-White	C-Honey	5	5	0	0,00794	0,0110278	*

C18.1.*n*.9.	C-White	C-Honey	5	5	0	0,00794	0,0110278	*
C18.1.*n*.7.	C-White	C-Honey	5	5	0	0,00794	0,0110278	*
C18.2.*n*.6.	C-White	C-Honey	5	5	25	0,00794	0,0110278	*
C18.3.*n*.3.	C-White	C-Honey	5	5	0	0,00794	0,0110278	*
C20.0	C-White	C-Honey	5	5	0	0,00794	0,0110278	*
MUFA	C-White	C-Honey	5	5	0	0,00794	0,0110278	*
*n*3	C-White	C-Honey	5	5	0	0,00794	0,0110278	*
*n*6	C-White	C-Honey	5	5	25	0,00794	0,0110278	*
*n*6/*n*3	C-White	C-Honey	5	5	25	0,00794	0,0110278	*
AI	C-White	C-Honey	5	5	25	0,00794	0,0110278	*
TI	C-White	C-Honey	5	5	25	0,00794	0,0110278	*
hH	C-White	C-Honey	5	5	0	0,00794	0,0110278	*
PI	C-White	C-Honey	5	5	0	0,00794	0,0110278	*
C14.0	C-White	C-Honey	5	5	1	0,0159	0,0209211	*
SFA	C-White	C-Honey	5	5	23	0,0317	0,039625	*
C18.0	C-White	C-Honey	5	5	20	0,151	0,1715909	ns
NVI	C-White	C-Honey	5	5	5	0,151	0,1715909	ns
C16.1.*n*.9.	C-White	C-Honey	5	5	16	0,548	0,5708333	ns
PUFA	C-White	C-Honey	5	5	9	0,548	0,5708333	ns
C15.0	C-White	C-Honey	5	5	12	1	1	ns

Abbreviations: ∑ PUFA, sum of polyunsaturated fatty acids; ∑ MUFA, sum of mono-unsaturated fatty acids; ∑ SFA, sum of saturated fatty acids; h/H ratio, hypocholesterolemic/hypercholesterolemic indices; AI, atherogenic index; TI, thrombogenic index; NVI, nutritive value index; PI, peroxidation index; *Statistically significant differences between mutants of *C. vulgaris* (*Wilcoxon rank sum test*, $q < 0.05$).

Table S4.3. Comparison of the fatty acid profile of extracts from C-Auto and C-Hetero with the mutant strains C-Honey and C-White (expressed as µg FA/mg dry biomass). Lipidic quality indexes are also shown. Data are presented as mean ± standard deviation (SD) ($n = 5$).

Fatty acid	**C-Auto**	**C-Hetero**	**C-Honey**	**C-White**
C14:0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
C15:0	0.1±0.3	0.0±0.0	0.0±0.0	0.0±0.0
C16:0	7.3±1.4	8.4±0.6	7.0±2.1	3.9±0.5
C16:1 (n-9)	0.5±0.1	0.8±0.1	0.8±0.2	0.4±0.1
C16:1 (n-7)	1.4±0.2	0.0±0.0	0.0±0.0	0.0±0.0
C16:2 (n-6)	3.5±0.6	2.6±0.2	1.1±0.4	1.3±0.2
C17:0	0.3±0.7	0.1±0.0	0.2±0.2	0.0±0.6
C16:3 (n-3)	7.1±1.0	1.2±0.1	1.4±0.5	0.1±0.0
C18:0	2.3±0.7	1.7±0.6	2.3±0.7	1.7±0.6
C18:1 (n-9)	1.8±0.5	5.3±0.5	4.6±1.4	2.0±0.2
C18:1 (n-7)	1.0±0.2	0.1±0.0	0.0±0.0	0.0±0.0
C18:2 (n-6)	8.2±1.5	11.1±0.7	6.8±2.2	4.3±0.5
C18:3 (n-3)	13.0±2.0	2.6±0.2	2.7±0.9	0.3±0.0
C20:0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Σ SFA	9.9±1.9	10.2±0.5	14.5±2.8	5.6±1.1
Σ MUFA	4.7±1.0	6.3±0.5	8.1±1.7	2.4±0.2
Σ PUFA	31.8±5.1	17.4±1.7	18.6±3.9	6.1±0.7
Σ (*n*-3)	20.1±3.0	3.8±0.3	6.4±1.4	0.5±0.1
Σ (*n*-6)	11.7±2.1	13.6±0.9	12.3±2.6	5.6±0.6
***n*-6/*n*-3 ratio**	0.6±0.0	3.6±0.0	1.9±0.0	12.6±0.5

AI	0.2±0.0	0.3±0.0	0.4±0.0	0.4±0.0
TI	0.1±0.0	0.5±0.0	0.5±0.0	1.0±0.2
h/H	4.9±0.2	2.8±0.0	2.4±0.0	2.1±0.1
NVI	0.7±0.1	0.9±0.1	1.0±0.0	0.9±0.1
PI	52.0±8.2	1.4±6.6	25.2±5.3	6.6±0.8

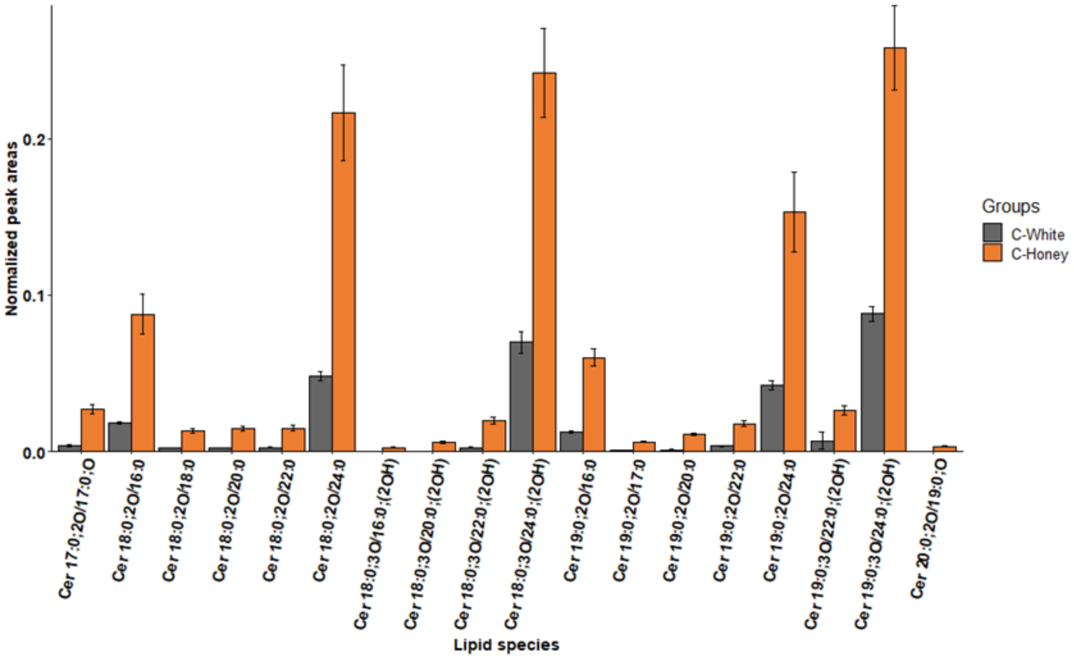


Figure S4.2. Ceramide (Cer) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments (n = 5).

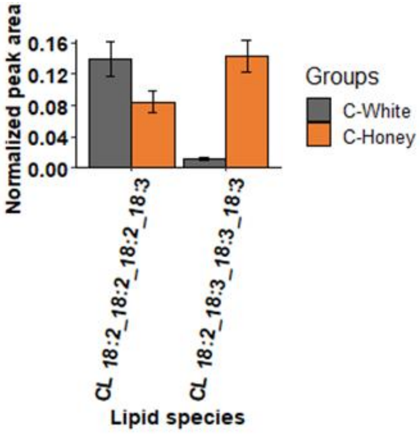


Figure S4.3. Cardiolipin (CL) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments (n = 5).

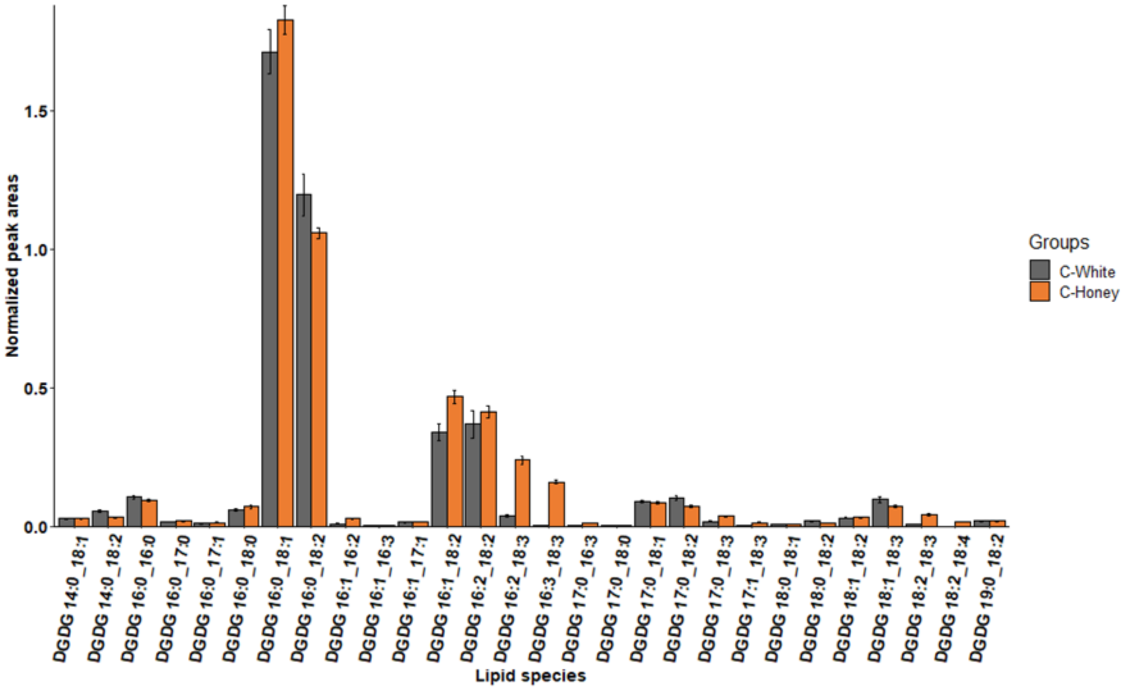


Figure S4.4. Digalactosyldiacylglycerol (DGDG) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments (n = 5).

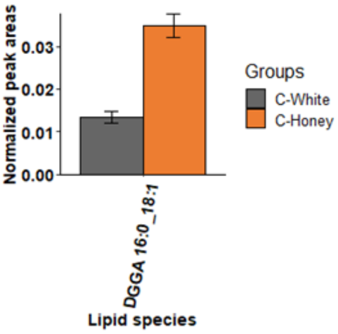


Figure S4.5. Diacylglycerylglucuronide (DGGA) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments (n = 5).

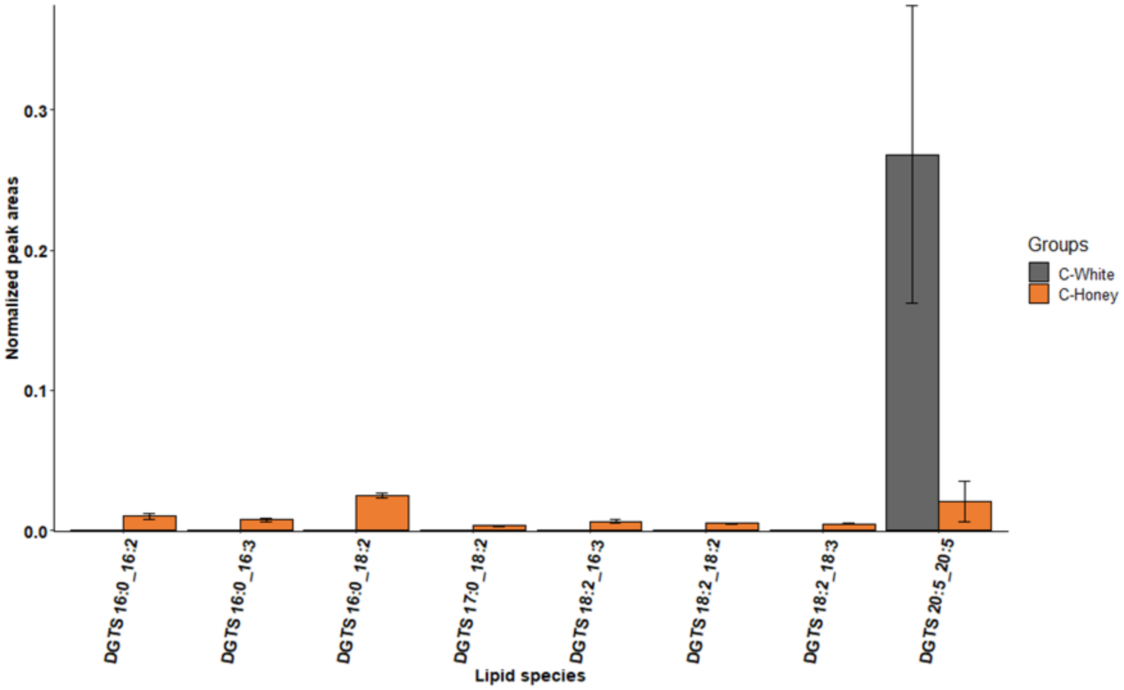


Figure S4.6. Diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine (DGTS) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments.

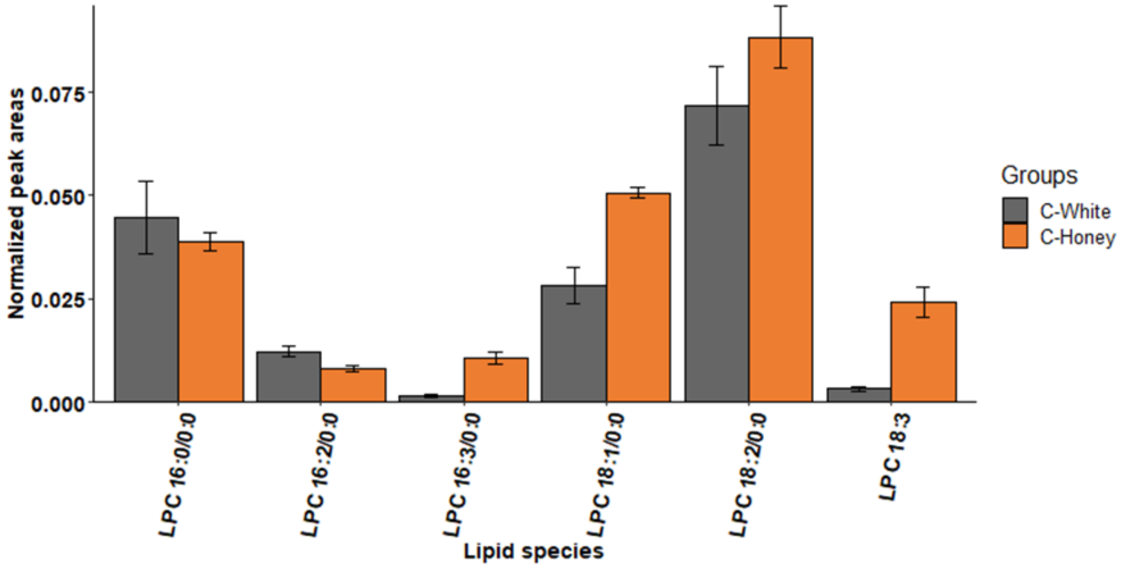


Figure S4.7. Lysophosphatidylcholine (LPC) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments.

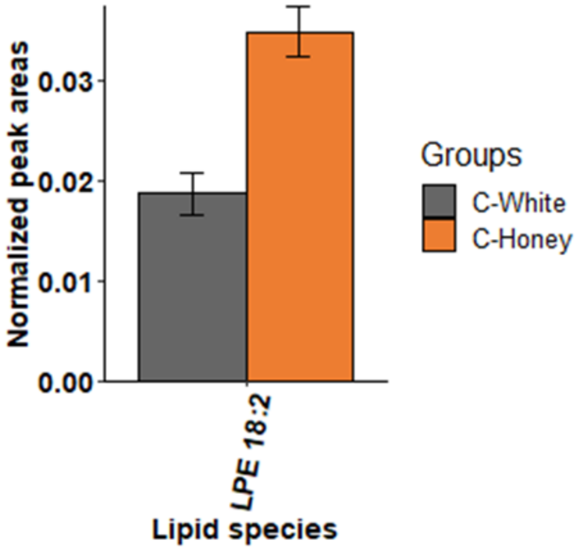


Figure S4.8. Lysophosphatidylethanolamine (LPE) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments.

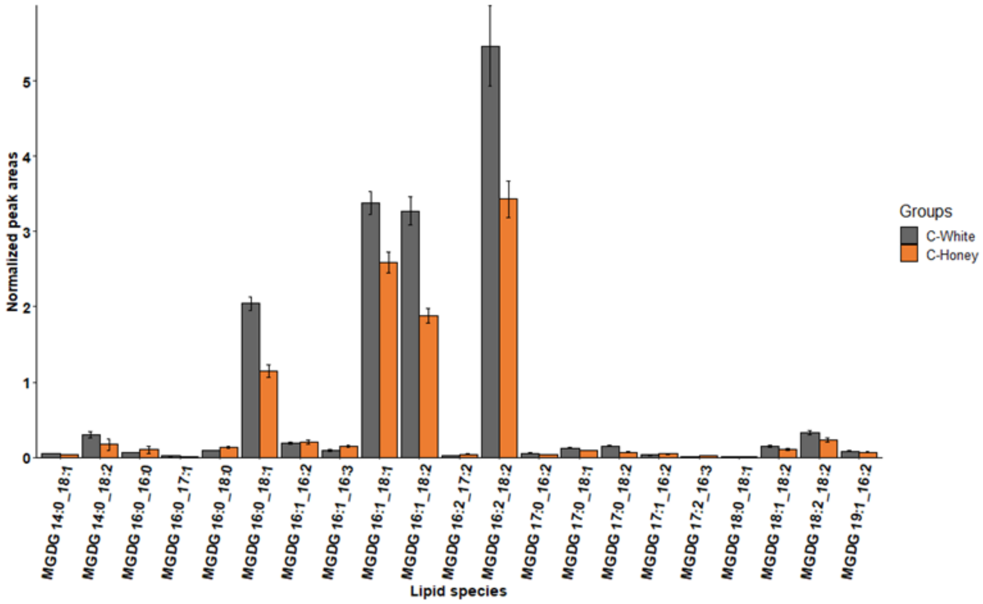


Figure S4.9. Monogalactosyldiacylglycerol (MGDG) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments.

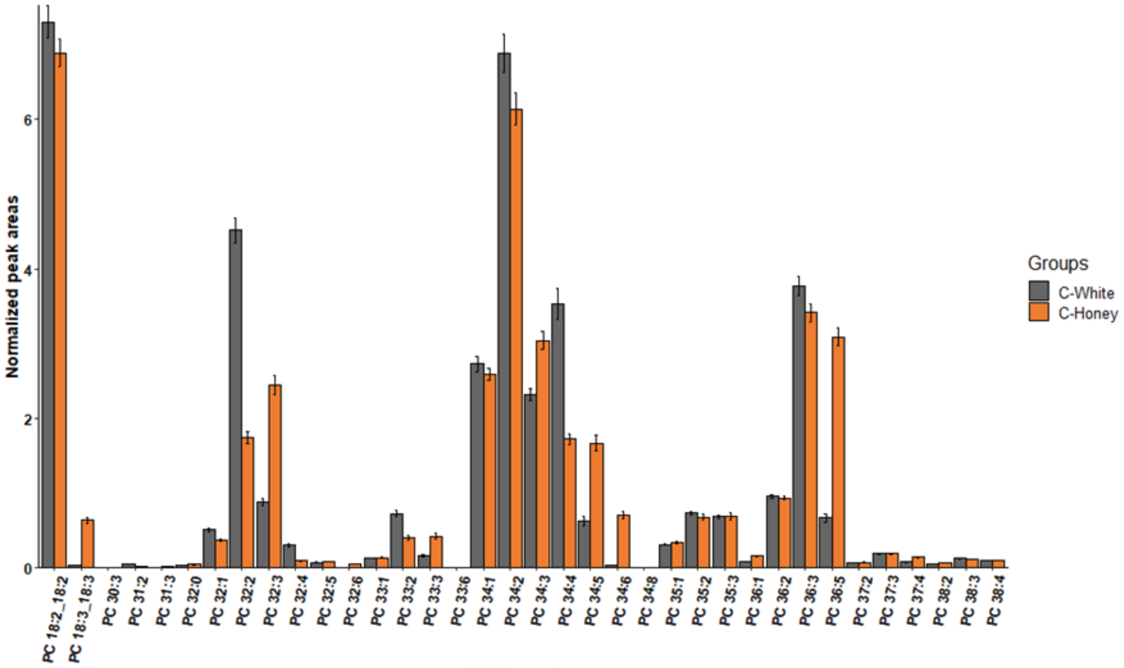


Figure S4.10. Phosphatidylcholine (PC) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments.

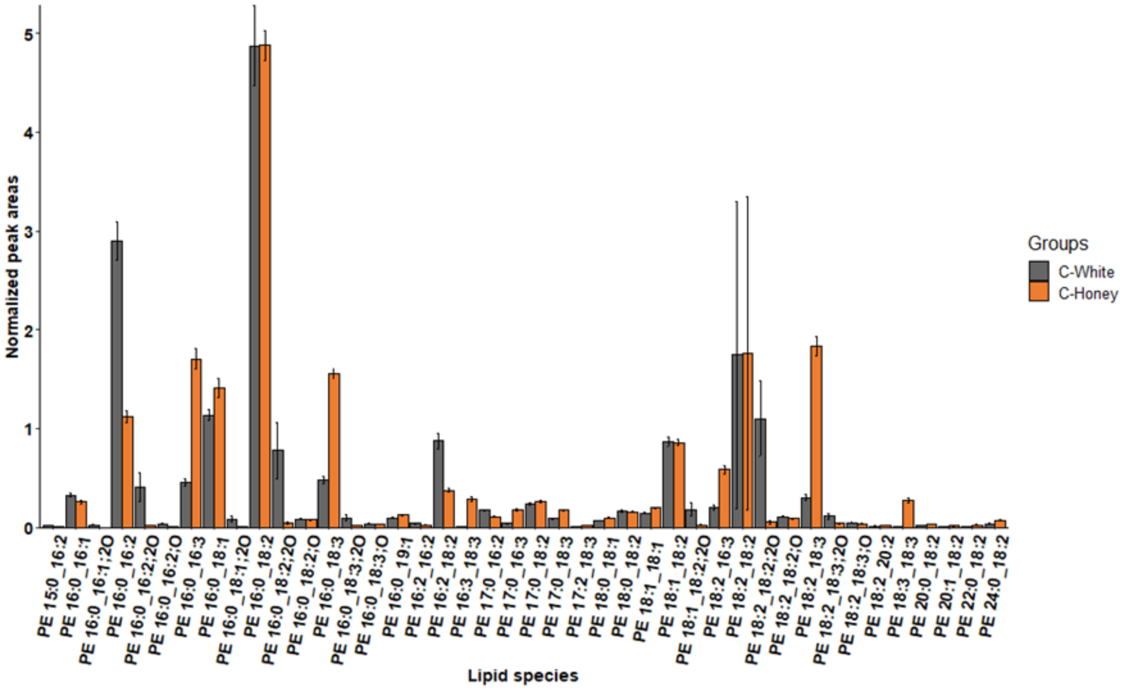


Figure S4.11. Phosphatidylethanolamine (PE) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments.

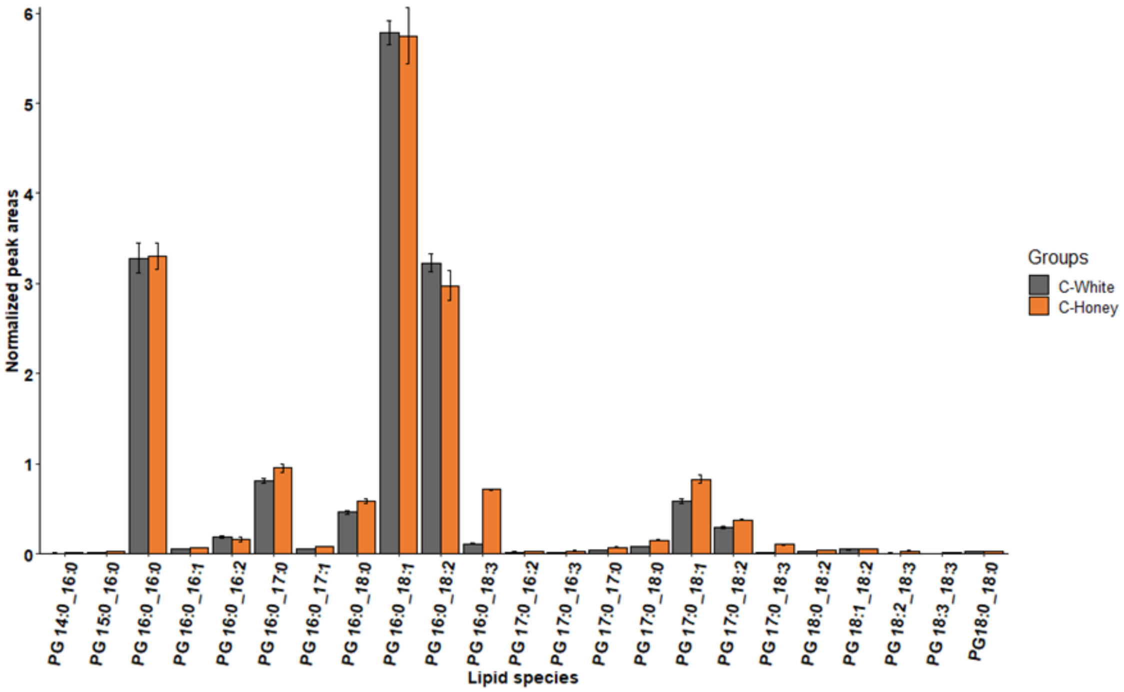


Figure S4.12. Phosphatidylglycerol (PG) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments.

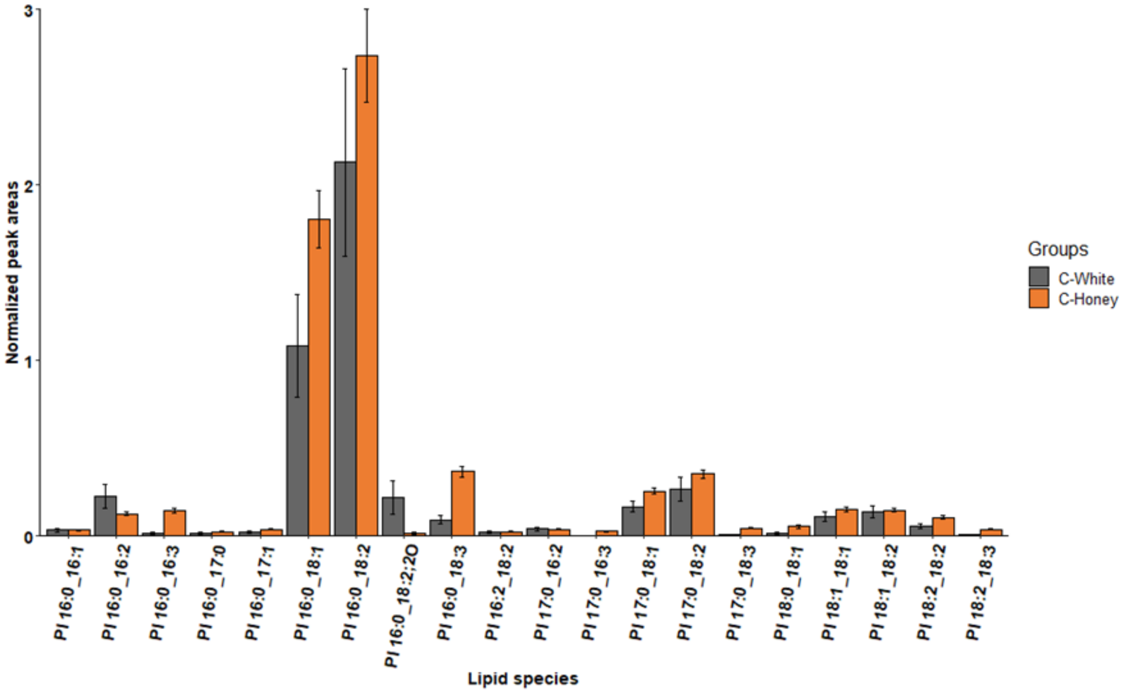


Figure S4.13. Phosphatidylinositol (PI) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments.

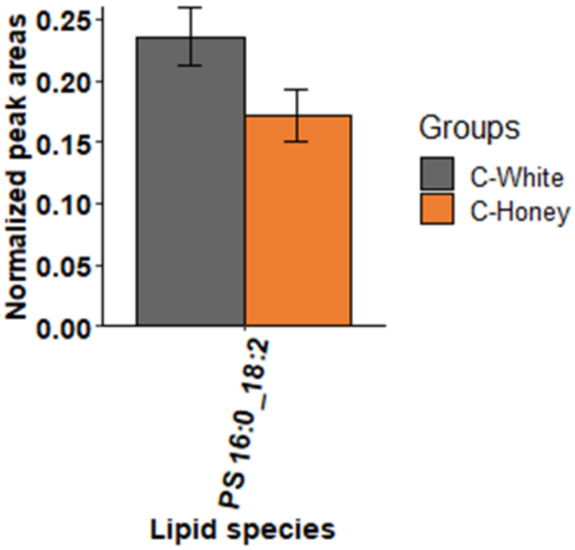


Figure S4.14. Phosphatidylserine (PS) specie identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments.

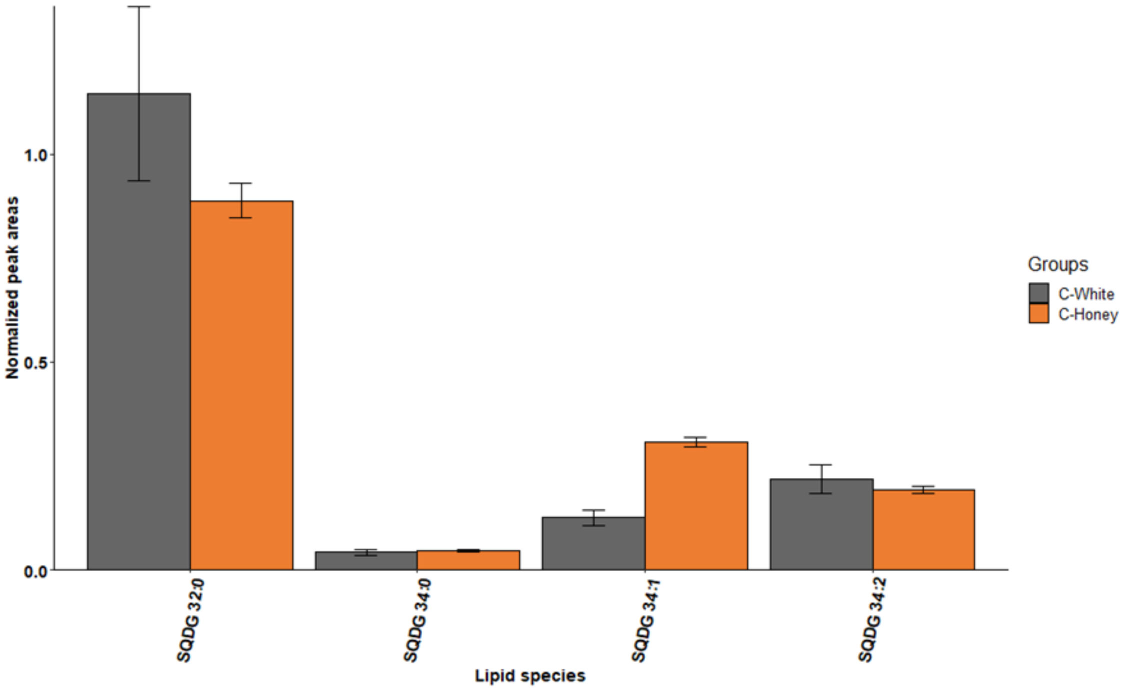


Figure S4.15. Sulfoquinovosyldiacylglycerol (SQDG) species identified in the total lipid extracts of C-Honey (orange color) and C-White (grey color) *C. vulgaris*, based on LC-MS/MS. Lipid species are represented by AAAA C:N (lipid class abbreviation, followed the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains). Data are represented as the mean ± standard deviation of five independent experiments.

Table S4.4. Exact mass measurements of the all lipid species identified by LC−MS in lipid extracts of C-Honey and C-White. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains.

*Lipid species identified for the first time in *C. vulgaris*.

Lipid species C:N	**Formula**	**Calculated *m/z***	**Observed *m/z***	**Error (ppm)**
PC identified as $[M+H]^+$				
PC 30:3	C38H70NO8P	700,49121	700,4924	-1,6988
*PC 31:2	C39H74NO8P	716,52252	716,5225	0,0279
*PC 31:3	C39H72NO8P	714,50677	714,5071	-0,4619
PC 32:0	C40H80NO8P	734,5694	734,5698	-0,5445
PC 32:1	C40H78NO8P	732,55377	732,5562	-3,3172
PC 32:2	C40H76NO8P	730,53809	730,5392	-1,5194
PC 32:3	C40H74NO8P	728,52252	728,5255	-4,0905
PC 32:4	C40H72NO8P	726,50677	726,5066	0,234
PC 32:5	C40H70NO8P	724,49121	724,4909	0,4279
PC 32:6	C40H68NO8P	722,47552	722,4771	-2,1869
*PC 33:1	C41H80NO8P	746,5694	746,5711	-2,2771
PC 33:2	C41H78NO8P	744,55377	744,5533	0,6313
PC 33:3	C41H76NO8P	742,53809	742,5387	-0,8215
*PC 33:6	C41H70NO8P	736,49121	736,4921	-1,2084
PC 34:1	C42H82NO8P	760,58508	760,5868	-2,2614
PC 34:2	C42H80NO8P	758,5694	758,5711	-2,2411
PC 34:3	C42H78NO8P	756,55377	756,5554	-2,1545
PC 34:4	C42H76NO8P	754,53809	754,5394	-1,7362
PC 34:5	C42H74NO8P	752,52252	752,5252	-3,5614
PC 34:6	C42H72NO8P	750,50677	750,5081	-1,7721
PC 34:8	C42H68NO8P	746,47552	746,4743	1,6343
PC 35:1	C43H84NO8P	774,60071	774,6017	-1,2781
PC 35:2	C43H82NO8P	772,58508	772,5844	0,8802
PC 35:3	C43H80NO8P	770,5694	770,5721	-3,5039
PC 36:1	C44H86NO8P	788,61639	788,6181	-2,1684
PC 36:2	C44H84NO8P	786,60071	786,6009	-0,2415
PC 36:3	C44H82NO8P	784,58508	784,5855	-0,5353
PC 36:4|PC 18:2_18:2	C44H80NO8P	782,5694	782,5705	-1,4056
PC 36:5	C44H78NO8P	780,55377	780,5533	0,6021
PC 36:6 PC 18:3_18:3	C44H76NO8P	778,53809	778,5393	-1,5542

PC 37:2	C45H86NO8P	800,61639	800,6155	1,1116
PC 37:3	C45H84NO8P	798,60071	798,5997	1,2647
PC 37:4	C45H82NO8P	796,58508	796,5855	-0,5273
PC 38:2	C46H88NO8P	814,63202	814,6325	-0,5892
PC 38:3	C46H86NO8P	812,61639	812,6185	-2,5966
PC 38:4	C46H84NO8P	810,60071	810,6017	-1,2213
LPC identified as [M+H]$^+$				
LPC 16:0/0:0	C24H50NO7P	496,3398	496,3411	-2,6192
LPC 16:2/0:0	C24H46NO7P	492,3085	492,3098	-2,6406
LPC 16:3/0:0	C24H44NO7P	490,2928	490,2946	-3,6713
LPC 18:1/0:0	C26H52NO7P	522,3554	522,3568	-2,6802
LPC 18:2/0:0	C26H50NO7P	520,3398	520,3414	-3,0749
LPC 18:3	C26H48NO7P	518,3241	518,3245	-0,7717
PE identified as [M-H]$^-$				
*PE 31:2|PE 15:0_16:2	C36H68NO8P	672,461	672,4609	0,1487
*PE 32:1;2O|PE 16:0_16:1;2O	C37H72NO10P	720,4821	720,482	0,1388
PE 32:1|PE 16:0_16:1	C37H72NO8P	688,4923	688,4938	-2,1787
*PE 32:2;2O|PE 16:0_16:2;2O	C37H70NO10P	718,4665	718,468	-2,0878
*PE 32:2;O|PE 16:0_16:2;O	C37H70NO9P	702,4715	702,4734	-2,7047
PE 32:2|PE 16:0_16:2	C37H70NO8P	686,4766	686,4776	-1,4567
PE 32:3|PE 16:0_16:3	C37H68NO8P	684,461	684,4612	-0,2922
PE 32:4|PE 16:2_16:2	C37H66NO8P	682,4453	682,4458	-0,7327
*PE 33:2|PE 17:0_16:2	C38H72NO8P	700,4923	700,4924	-0,1428
PE 33:3|PE 17:0_16:3	C38H70NO8P	698,4766	698,477	-0,5727
*PE 34:1;2O|PE 16:0_18:1;2O	C39H76NO10P	748,5134	748,514	-0,8016
PE 34:1|PE 16:0_18:1	C39H76NO8P	716,5236	716,5244	-1,1165
*PE 34:2;2O|PE 16:0_18:2;2O	C39H74NO10P	746,4978	746,4994	-2,1433
*PE 34:2; O|PE 16:0_18:2;O	C39H74NO9P	730,5028	730,5037	-1,232
PE 34:2|PE 16:0_18:2	C39H74NO8P	714,5079	714,5046	4,6186
*PE 34:3;2O|PE 16:0_18:3;2O	C39H72NO10P	744,4821	744,482	0,1343
*PE 34:3;O|PE 16:0_18:3;O	C39H72NO9P	728,4872	728,4885	-1,7845
PE 34:3|PE 16:0_18:3	C39H72NO8P	712,4923	712,4929	-0,8421
PE 34:4|PE 16:2_18:2	C39H70NO8P	710,4766	710,4772	-0,8445
PE 34:5|PE 18:2_16:3	C39H68NO8P	708,461	708,4617	-0,9881
PE 34:6|PE 16:3_18:3	C39H66NO8P	706,4453	706,446	-0,9909
*PE 35:1|PE 16:0_19:1	C40H78NO8P	730,5392	730,5409	-2,327
PE 35:2|PE 17:0_18:2	C40H76NO8P	728,5236	728,523	0,8236

PE 35:3|PE 17:0_18:3	C40H74NO8P	726,5079	726,5085	-0,8259
*PE 35:5|PE 17:2_18:3	C40H72NO9P	722,4766	722,4768	-0,2768
*PE 36:1|PE 18:0_18:1	C41H80NO8P	744,5549	744,5544	0,6715
PE 36:2|PE 18:0_18:2	C41H78NO8P	742,5392	742,5403	-1,4814
PE 36:2|PE 18:1_18:1	C41H78NO8P	742,5392	742,5404	-1,6161
*PE 36:3;2O|PE 18:1_18:2;2O	C41H76NO10P	772,5134	772,5136	-0,2589
PE 36:3|PE 18:1_18:2	C41H76NO8P	740,5236	740,5242	-0,8102
*PE 36:4;2O|PE 18:2_18:2;2O	C41H74NO10P	770,4978	770,4965	1,6872
PE 36:4;O|PE 18:2_18:2;O	C41H74NO9P	754,5028	754,5033	-0,6627
PE 36:4|PE 18:2_18:2	C41H74NO8P	738,5079	738,5125	-3,3852
*PE 36:5;2O|PE 18:2_18:3;2O	C41H72NO10P	768,4821	768,4822	-0,1301
*PE 36:5;O|PE 18:2_18:3;O	C41H72NO9P	752,4872	752,4878	-0,7974
PE 36:5|PE 18:2_18:3	C41H72NO8P	736,4923	736,4914	1,222
PE 36:6|PE 18:3_18:3	C41H70NO8P	734,4766	734,4769	-0,4085
*PE 38:2|PE 20:0_18:2	C43H82NO8P	770,5705	770,5722	-2,2062
*PE 38:3|PE 20:1_18:2	C43H80NO8P	768,5549	768,5576	-3,5131
*PE 38:4|PE 18:2_20:2	C43H78NO8P	766,5392	766,5407	-1,9568
*PE 40:2|PE 22:0_18:2	C45H86NO8P	798,6018	798,604	-2,7548
*PE 42:2|PE 24:0_18:2	C47H90NO8P	826,6331	826,6359	-3,3872
LPE identified as [M-H]$^-$				
LPE 18:2	C23H44NO7P	476,2783	476,2782	0,21
PG identified as [M-H]$^-$				
*PG 30:0|PG 14:0_16:0	C36H71O10P	693,4712	693,4713	-0,1442
*PG 31:0|PG 15:0_16:0	C37H73O10P	707,4869	707,4875	-0,8481
PG 32:0|PG 16:0_16:0	C38H75O10P	721,5025	721,5031	-0,8316
PG 32:1|PG 16:0_16:1	C38H73O10P	719,4869	719,4854	2,0848
*PG 32:2|PG 16:0_16:2	C38H71O10P	717,4712	717,4707	0,6969
PG 33:0|PG 16:0_17:0	C39H77O10P	735,5182	735,5193	-1,4955
*PG 33:1|PG 16:0_17:1	C39H75O10P	733,5025	733,5042	-2,3176
*PG 33:2|PG 17:0_16:2	C39H73O10P	731,4869	731,4878	-1,2304
*PG 33:3|PG 17:0_16:3	C39H71O10P	729,4712	729,4704	1,0967
PG 34:0|PG 16:0_18:0	C40H79O10P	749,5338	749,5353	-2,0012
PG 34:0|PG 17:0_17:0	C40H79O10P	749,5338	749,5346	-1,0673
PG 34:1|PG 16:0_18:1	C40H77O10P	747,5182	747,5186	-0,5351
PG 34:2|PG 16:0_18:2	C40H75O10P	745,5025	745,5029	-0,5366
PG 34:3|PG 16:0_18:3	C40H73O10P	743,4869	743,4864	0,6725
*PG 35:0|PG 17:0_18:0	C41H81O10P	763,5495	763,5504	-1,1787

*PG 35:1|PG 17:0_18:1	C41H79O10P	761,5338	761,5341	-0,3939
*PG 35:2|PG 17:0_18:2	C41H77O10P	759,5182	759,5184	-0,2633
*PG 35:3|PG 17:0_18:3	C41H75O10P	757,5025	757,5041	-2,1122
PG 36:0|PG18:0_18:0	C42H83O10P	777,5651	777,5675	-3,0866
PG 36:2|PG 18:0_18:2	C42H79O10P	773,5338	773,5346	-1,0342
PG 36:3|PG 18:1_18:2	C42H77O10P	771,5182	771,5171	1,4258
PG 36:5|PG 18:2_18:3	C42H73O10P	767,4869	767,4883	-1,8241
*PG 36:6|PG 18:3_18:3	C42H71O10P	765,4712	765,4708	0,5226
PI identified as $[M-H]^-$				
*PI 32:1|PI 16:0_16:1	C41H77O13P	807,5029	807,5019	1,2384
PI 32:2|PI 16:0_16:2	C41H75O13P	805,4873	805,4866	0,869
*PI 32:3|PI 16:0_16:3	C41H73O13P	803,4716	803,4719	-0,3734
*PI 33:0|PI 16:0_17:0	C42H81O13P	823,5342	823,5348	-0,7286
*PI 33:1|PI 16:0_17:1	C42H79O13P	821,5186	821,5187	-0,1217
*PI 33:2|PI 17:0_16:2	C42H77O13P	819,5029	819,5041	-1,4643
*PI 33:3|PI 17:0_16:3	C42H75O13P	817,4873	817,4877	-0,4893
PI 34:1|PI 16:0_18:1	C43H81O13P	835,5342	831,4701	0
*PI 34:2;2O|PI 16:0_18:2;2O	C43H79O15P	865,5084	835,5342	2,0645
PI 34:2|PI 16:0_18:2	C43H79O13P	833,5186	833,5186	0
PI 34:3|PI 16:0_18:3	C43H77O13P	831,5029	831,5037	-0,9621
*PI 34:4|PI 16:2_18:2	C43H75O13P	829,4873	829,4899	-3,1345
*PI 35:1|PI 17:0_18:1	C44H83O13P	849,5499	849,5491	0,9417
PI 35:2|PI 17:0_18:2	C44H81O13P	847,5342	847,5352	-1,1799
*PI 35:3|PI 17:0_18:3	C44H79O13P	845,5186	845,5204	-2,1289
*PI 36:1|PI 18:0_18:1	C45H85O13P	863,5655	863,5656	-0,1158
PI 36:2|PI 18:1_18:1	C45H83O13P	861,5499	861,5482	1,9732
PI 36:3|PI 18:1_18:2	C45H81O13P	859,5342	859,5359	-1,9778
PI 36:4|PI 18:2_18:2	C45H79O13P	857,5186	857,5209	-2,6822
PI 36:5|PI 18:2_18:3	C45H77O13P	855,5029	855,5039	-1,1689
PS identified as $[M-H]^-$				
PS 34:2|PS 16:0_18:2	C40H74NO10P	760,5123	760,5135	-1,5779
CL identified as $[M-H]^-$				
*CL 72:11|CL 18:2_18:3_18:3_18:3	C81H136O17P2	1441,918	1441,921	-2,0806
*CL 72:9|CL 18:2_18:2_18:2_18:3	C81H140O17P2	1445,949	1445,944	3,4579

Cer identified as $[M+H]^+$				
*Cer 34:0;2O|Cer 18:0;2O/16:0	$C_{34}H_{69}NO_3$	540,535	540,5365	-2,775
*Cer 34:0;3O|Cer 17:0;2O/17:0; O	$C_{34}H_{69}NO_4$	556,5299	556,5302	-0,5391
*Cer 34:0;4O|Cer 18:0;3O/16:0;(2OH)	$C_{34}H_{69}NO_5$	572,5249	572,5262	-2,2706
Cer 35:0;2O|Cer 19:0;2O/16:0	$C_{35}H_{71}NO_3$	554,5507	554,5523	-2,8852
*Cer 36:0;2O|Cer 18:0;2O/18:0	$C_{36}H_{73}NO_3$	568,5663	568,5665	-0,3518
*Cer 36:0;2O|Cer 19:0;2O/17:0	$C_{36}H_{73}NO_3$	568,5663	568,5665	-0,3518
* Cer 38:0;2O|Cer 18:0;2O/20:0	$C_{38}H_{77}NO_3$	596,5976	596,5983	-1,1733
* Cer 38:0;4O|Cer 18:0;3O/20:0;(2OH)	$C_{38}H_{77}NO_5$	628,5875	628,5899	-3,8181
* Cer 39:0;2O|Cer 19:0;2O/20:0	$C_{39}H_{79}NO_3$	610,6133	610,6143	-1,6377
* Cer 39:0;3O|Cer 20:0;2O/19:0; O	$C_{39}H_{79}NO_4$	626,6082	626,609	-1,2767
* Cer 40:0;2O|Cer 18:0;2O/22:0	$C_{40}H_{81}NO_3$	624,6289	624,63	-1,761
* Cer 40:0;4O|Cer 18:0;3O/22:0;(2OH)	$C_{40}H_{81}NO_5$	656,6188	656,6208	-3,0459
*Cer 41:0;2O|Cer 19:0;2O/22:0	$C_{41}H_{83}NO_3$	638,6446	638,6447	-0,1566
*Cer 41:0;4O|Cer 19:0;3O/22:0;(2OH)	$C_{41}H_{83}NO_5$	670,6344	670,6371	-4,026
*Cer 42:0;2O|Cer 18:0;2O/24:0	$C_{42}H_{85}NO_3$	652,6602	652,662	-2,7579
*Cer 42:0;4O|Cer 18:0;3O/24:0;(2OH)	$C_{42}H_{85}NO_4$	668,6551	668,6568	-2,5424
*Cer 43:0;2O|Cer 19:0;2O/24:0	$C_{42}H_{85}NO_5$	684,6501	684,6509	-1,1685
*Cer 43:0;4O|Cer 19:0;3O/24:0;(2OH)	$C_{43}H_{87}NO_3$	666,6759	666,6782	-3,45
MGDG identified as [M+HCOO]-				
*MGDG 32:0|MGDG 16:0_16:0	$C_{41}H_{78}O_{10}$	775,5577	775,5597	-2,5788
*MGDG 32:1|MGDG 14:0_18:1	$C_{41}H_{76}O_{10}$	773,5421	773,5426	-0,6464
MGDG 32:2|MGDG 14:0_18:2	$C_{41}H_{74}O_{10}$	771,5264	771,5267	-0,3888
MGDG 32:3|MGDG 16:1_16:2	$C_{41}H_{72}O_{10}$	769,5108	769,5108	0
MGDG 32:4|MGDG 16:1_16:3	$C_{41}H_{70}O_{10}$	767,4951	767,4947	0,5212
*MGDG 33:1|MGDG 16:0_17:1	$C_{42}H_{78}O_{10}$	787,5577	787,5591	-1,7776
*MGDG 33:2|MGDG 17:0_16:2	$C_{42}H_{76}O_{10}$	785,5421	785,545	-3,6917
*MGDG 33:3|MGDG 17:1_16:2	$C_{42}H_{74}O_{10}$	783,5264	783,5283	-2,4249
*MGDG 33:4|MGDG 16:2_17:2	$C_{42}H_{72}O_{10}$	781,5108	781,5104	0,5118

*MGDG 33:5|MGDG 17:2_16:3	C42H70O10	779,4951	779,4941	1,2829
MGDG 34:0|MGDG 16:0_18:0	C43H82O10	803,589	803,5879	1,3689
MGDG 34:1|MGDG 16:0_18:1	C43H80O10	801,5734	801,5745	-1,3723
MGDG 34:2|MGDG 16:1_18:1	C43H78O10	799,5577	799,5587	-1,2507
MGDG 34:3|MGDG 16:1_18:2	C43H76O10	797,5421	797,5422	-0,1254
MGDG 34:4|MGDG 16:2_18:2	C43H74O10	795,5264	795,5266	-0,2514
MGDG 35:1|MGDG 17:0_18:1	C44H82O10	815,589	815,5915	-3,0653
*MGDG 35:2|MGDG 17:0_18:2	C44H80O10	813,5734	813,5743	-1,1062
*MGDG 35:3|MGDG 19:1_16:2	C44H78O10	811,5577	811,5586	-1,109
*MGDG 36:1|MGDG 18:0_18:1	C45H84O10	829,6047	829,6055	-0,9643
MGDG 36:3|MGDG 18:1_18:2	C45H80O10	825,5734	825,573	0,4845
MGDG 36:4|MGDG 18:2_18:2	C45H78O10	823,5577	823,5575	0,2428
DGDG identified as $[M+NH_4]^+$				
DGDG 32:0|DGDG 16:0_16:0	C47H88O15	910,6461	910,6459	0,2196
DGDG 32:1|DGDG 14:0_18:1	C47H86O15	908,6305	908,6286	2,0911
DGDG 32:2|DGDG 14:0_18:2	C47H84O15	906,6148	906,6139	0,9927
DGDG 32:3|DGDG 16:1_16:2	C47H82O15	904,5992	904,6018	-2,8742
DGDG 32:4|DGDG 16:1_16:3	C47H80O15	902,5835	902,5865	-3,3238
*DGDG 33:0|DGDG 16:0_17:0	C48H90O15	924,6618	924,663	-1,2978
*DGDG 33:1|DGDG 16:0_17:1	C48H88O15	922,6461	922,6471	-1,0838
*DGDG 33:2|DGDG 16:1_17:1	C48H86O15	920,6305	920,6304	0,1086
*DGDG 33:3|DGDG 17:0_16:3	C48H84O15	918,6148	918,6139	0,9797
*DGDG 34:0|DGDG 16:0_18:0	C49H92O15	938,6774	938,6789	-1,598
DGDG 34:1|DGDG 16:0_18:1	C49H90O15	936,6618	936,6615	0,3203
DGDG 34:2|DGDG 16:0_18:2	C49H88O15	934,6461	934,6458	0,321
DGDG 34:3|DGDG 16:1_18:2	C49H86O15	932,6305	932,6318	-1,3939
DGDG 34:4|DGDG 16:2_18:2	C49H84O15	930,6148	930,618	-3,4386
DGDG 34:5|DGDG 16:2_18:3	C49H82O15	928,5992	928,6028	-3,8768

DGDG 34:6|DGDG 16:3_18:3	C49H80O15	926,5835	926,5863	-3,0219
*DGDG 35:0|DGDG 17:0_18:0	C50H94O15	952,6931	952,6924	0,7348
DGDG 35:1|DGDG 17:0_18:1	C50H92O15	950,6774	950,68	-2,7349
DGDG 35:2|DGDG 17:0_18:2	C50H90O15	948,6618	948,6611	0,7379
DGDG 35:3|DGDG 17:0_18:3	C50H88O15	946,6461	946,6478	-1,7958
*DGDG 35:4|DGDG 17:1_18:3	C50H86O15	944,6305	944,6313	-0,8469
*DGDG 36:1|DGDG 18:0_18:1	C51H94O15	964,6931	964,6926	0,5183
DGDG 36:2|DGDG 18:0_18:2	C51H92O15	962,6774	962,6768	0,6233
DGDG 36:3|DGDG 18:1_18:2	C51H90O15	960,6618	960,6617	0,1041
DGDG 36:4|DGDG 18:1_18:3	C51H88O15	958,6461	958,6458	0,3129
DGDG 36:5|DGDG 18:2_18:3	C51H86O15	956,6305	956,6295	1,0453
DGDG 36:6|DGDG 18:2_18:4	C51H84O15	954,6148	954,6144	0,419
*DGDG 37:2|DGDG 19:0_18:2	C52H94O15	976,6931	976,6947	-1,6382
DGGA identified as $[M-H]^-$				
*DGGA 34:1|DGGA 16:0_18:1	C43H78O11	769,5471	769,5481	-1,2995
SQDG identified as $[M-H]^-$				
SQDG 32:0	C41H78O12S	793,5141	793,5135	0,7561
SQDG 34:0	C43H82O12S	821,5454	821,5463	-1,0955
SQDG 34:1	C43H80O12S	819,5298	819,5315	-2,0744
SQDG 34:2	C43H78O12S	817,5137	817,514	-0,367
DGTS identified as $[M+NH_4]^+$				
*DGTS 32:2|DGTS 16:0_16:2	C42H77NO7	708,5773	708,578	-0,9879
*DGTS 32:3|DGTS 16:0_16:3	C42H75NO7	706,5616	706,5604	1,6984
*DGTS 34:2|DGTS 16:0_18:2	C44H81NO7	736,6086	736,6115	-3,937
*DGTS 34:5|DGTS 18:2_16:3	C44H75NO7	730,5616	730,5622	-0,8213
*DGTS 35:2|DGTS 17:0_18:2	C45H83NO7	750,6242	750,6246	-0,5329
*DGTS 36:4|DGTS 18:2_18:2	C46H81NO7	760,6086	760,608	0,7888
*DGTS 36:5|DGTS 18:2_18:3	C46H79NO7	758,5929	758,5926	0,3955
*DGTS 40:10|DGTS 20:5_20:5	C50H77NO7	804,5773	804,5771	0,2486

Abbreviations: Cer, Ceramide; CL, Cardiolipin; DGDG, Digalactosyldiacylglycerol

DGGA, Diacylglycerylglucuronide; DGTS, Diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGDG, Monogalactosyldiacylglycerol; LPC, Lysophosphatidylcholine; LPE, Lysophosphatidylethanolamine; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; PI, Phosphatidylinositol; PS, Phosphatidylserine; SQDG, Sulfoquinovosyldiacylglycerol.

Table S4.5. Results of Wilcoxon rank-sum test (FDR adjusted) for statistically significant differences of polar lipids between C-Honey and C- White.

variable	group1	group2	n1	n2	statistic	p	p.adj	p.adj. signif
CL.18.2_18.3_ 18.3_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
CL.18.2_18.2_ 18.2_18.3	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
DGGA.16.0_18.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
LPE.18.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
MGDG.14.0_18.1	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
MGDG.16.1_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
MGDG.17.0_16.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
MGDG.17.1_16.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
MGDG.16.2_17.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
MGDG.17.2_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
MGDG.16.0_18.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
MGDG.16.0_18.1	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
MGDG.16.1_18.1	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
MGDG.16.1_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
MGDG.16.2_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
MGDG.17.0_18.1	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
MGDG.17.0_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
MGDG.18.0_18.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
MGDG.18.1_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
MGDG.18.2_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.15.0_16.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.16.0_16.1.2O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.16.0_16.1	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.16.0_16.2.2O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.16.0_16.2.O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.16.0_16.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.16.0_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.16.2_16.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.17.0_16.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.17.0_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*

PE.16.0_18.1.2O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.16.0_18.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.16.0_18.2.2O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.16.0_18.3.2O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.16.0_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.16.2_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.18.2_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.16.3_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.16.0_19.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.17.0_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.17.2_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.18.0_18.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.18.1_18.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.18.1_18.2.2O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.18.2_18.2.2O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.18.2_18.3.2O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.18.2_18.3.O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PE.18.2_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.18.3_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.20.0_18.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.20.1_18.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.18.2_20.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE.22.0_18.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PE 24:0_18:2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.15.0_16.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.16.0_16.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.16.0_17.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.16.0_17.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.17.0_16.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.17.0_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.16.0_18.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.17.0_17.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.16.0_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.17.0_18.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.17.0_18.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.17.0_18.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.17.0_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.18.0_18.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.18.2_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PG.18.3_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*

PI.16.0_16.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PI.16.0_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PI.16.0_17.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PI.17.0_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PI.16.0_18.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PI.16.0_18.2.2O	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PI.16.0_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PI.17.0_18.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PI.17.0_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PI.18.0_18.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PI.18.2_18.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PI.18.2_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
SQDG.34.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.18.0.2O.16.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.17.0.2O.17.0.O	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.18.0.3O.16.0..2OH.	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.19.0.2O.16.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.18.0.2O.18.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.19.0.2O.17.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.18.0.2O.20.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.18.0.3O.20.0..2OH.	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.19.0.2O.20.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.20.0.2O.19.0.O	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.18.0.2O.22.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.18.0.3O.22.0..2OH.	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.19.0.2O.22.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.19.0.3O.22.0..2OH.	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.18.0.2O.24.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.18.0.3O.24.0..2OH.	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.19.0.2O.24.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
Cer.19.0.3O.24.0..2OH.	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.14.0_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
DGDG.16.1_16.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.16.1_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.16.0_17.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.16.0_17.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.17.0_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.16.0_18.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.16.0_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
DGDG.16.1_18.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*

DGDG.16.2_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.16.3_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.17.0_18.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.17.0_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
DGDG.17.0_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.17.1_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.18.0_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
DGDG.18.1_18.3	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
DGDG.18.2_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGDG.18.2_18.4	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGTS.16.0_16.2	C-White	C-Honey	5	5	0	0,00749	0,01073936	*
DGTS.16.0_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGTS.16.0_18.2	C-White	C-Honey	5	5	0	0,00749	0,01073936	*
DGTS.18.2_16.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGTS.17.0_18.2	C-White	C-Honey	5	5	0	0,00749	0,01073936	*
DGTS.18.2_18.2	C-White	C-Honey	5	5	0	0,00749	0,01073936	*
DGTS.18.2_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
DGTS.20.5_20.5	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
LPC.16.2.0.0	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
LPC.16.3.0.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
LPC.18.1.0.0	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
LPC.18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.30.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.31.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PC.31.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.32.1	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PC.32.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PC.32.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.32.4	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PC.32.6	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.33.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PC.33.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.33.6	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.34.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PC.34.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.34.4	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
PC.34.5	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.34.6	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.36.1	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.36.3	C-White	C-Honey	5	5	25	0,00794	0,01073936	*

PC.36.5	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.18.3_18.3	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.37.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.37.4	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PC.38.2	C-White	C-Honey	5	5	0	0,00794	0,01073936	*
PS.16.0_18.2	C-White	C-Honey	5	5	25	0,00794	0,01073936	*
MGDG.16.0_17.1	C-White	C-Honey	5	5	24	0,0159	0,02070926	*
MGDG.19.1_16.2	C-White	C-Honey	5	5	24	0,0159	0,02070926	*
PE.17.0_18.2	C-White	C-Honey	5	5	1	0,0159	0,02070926	*
DGDG.16.0_16.0	C-White	C-Honey	5	5	24	0,0159	0,02070926	*
DGDG.16.0_18.1	C-White	C-Honey	5	5	1	0,0159	0,02070926	*
PC.32.0	C-White	C-Honey	5	5	1	0,0159	0,02070926	*
PE.18.2_18.2.O	C-White	C-Honey	5	5	23	0,0317	0,03957811	*
PG.14.0_16.0	C-White	C-Honey	5	5	2	0,0317	0,03957811	*
PG.16.0_18.2	C-White	C-Honey	5	5	23	0,0317	0,03957811	*
SQDG.32.0	C-White	C-Honey	5	5	23	0,0317	0,03957811	*
PC.35.1	C-White	C-Honey	5	5	2	0,0317	0,03957811	*
PC.18.2_18.2	C-White	C-Honey	5	5	23	0,0317	0,03957811	*
PC.38.3	C-White	C-Honey	5	5	23	0,0317	0,03957811	*
PE.16.0_18.3.O	C-White	C-Honey	5	5	22	0,0556	0,06590787	ns
PI.16.0_17.0	C-White	C-Honey	5	5	3	0,0556	0,06590787	ns
PI.16.0_18.2	C-White	C-Honey	5	5	3	0,0556	0,06590787	ns
PI.17.0_18.2	C-White	C-Honey	5	5	3	0,0556	0,06590787	ns
PI.18.1_18.1	C-White	C-Honey	5	5	3	0,0556	0,06590787	ns
LPC.18.2.0.0	C-White	C-Honey	5	5	3	0,0556	0,06590787	ns
PC.32.5	C-White	C-Honey	5	5	3	0,0556	0,06590787	ns
PC.34.1	C-White	C-Honey	5	5	22	0,0556	0,06590787	ns
PC.35.2	C-White	C-Honey	5	5	22	0,0556	0,06590787	ns
MGDG.14.0_18.2	C-White	C-Honey	5	5	21	0,0952	0,10916957	ns
PG.16.0_16.2	C-White	C-Honey	5	5	21	0,0952	0,10916957	ns
PG.18.1_18.2	C-White	C-Honey	5	5	4	0,0952	0,10916957	ns
LPC.16.0.0.0	C-White	C-Honey	5	5	21	0,0952	0,10916957	ns
PC.33.1	C-White	C-Honey	5	5	4	0,0952	0,10916957	ns
PC.38.4	C-White	C-Honey	5	5	4	0,0952	0,10916957	ns
MGDG.16.0_16.0	C-White	C-Honey	5	5	5	0,151	0,16768947	ns
PE.16.0_18.2.O	C-White	C-Honey	5	5	20	0,151	0,16768947	ns
PE.18.0_18.2	C-White	C-Honey	5	5	20	0,151	0,16768947	ns
PI.16.2_18.2	C-White	C-Honey	5	5	5	0,151	0,16768947	ns
DGDG.16.2_18.2	C-White	C-Honey	5	5	5	0,151	0,16768947	ns
PC.34.8	C-White	C-Honey	5	5	20	0,151	0,16768947	ns

PG18.0_18.0	C-White	C-Honey	5	5	6	0,222	0,24396875	ns
DGDG.17.0_18.1	C-White	C-Honey	5	5	19	0,222	0,24396875	ns
SQDG.34.0	C-White	C-Honey	5	5	8	0,421	0,45321939	ns
SQDG.34.2	C-White	C-Honey	5	5	17	0,421	0,45321939	ns
DGDG.16.1_17.1	C-White	C-Honey	5	5	8	0,421	0,45321939	ns
PC.36.2	C-White	C-Honey	5	5	17	0,421	0,45321939	ns
PG.16.0_18.1	C-White	C-Honey	5	5	16	0,548	0,57814	ns
PI.16.0_16.1	C-White	C-Honey	5	5	9	0,548	0,57814	ns
PI.18.1_18.2	C-White	C-Honey	5	5	9	0,548	0,57814	ns
DGDG.18.1_18.2	C-White	C-Honey	5	5	9	0,548	0,57814	ns
MGDG.16.1_16.2	C-White	C-Honey	5	5	10	0,69	0,72074257	ns
PE.16.0_18.2	C-White	C-Honey	5	5	15	0,69	0,72074257	ns
PE.18.1_18.2	C-White	C-Honey	5	5	11	0,841	0,85725121	ns
PE.18.2_18.2	C-White	C-Honey	5	5	14	0,841	0,85725121	ns
DGDG.18.0_18.1	C-White	C-Honey	5	5	11	0,841	0,85725121	ns
DGDG.19.0_18.2	C-White	C-Honey	5	5	11	0,841	0,85725121	ns
PC.37.3	C-White	C-Honey	5	5	11	0,841	0,85725121	ns
PG.16.0_16.0	C-White	C-Honey	5	5	12	1	1	ns
PI.17.0_16.2	C-White	C-Honey	5	5	13	1	1	ns
DGDG.14.0_18.1	C-White	C-Honey	5	5	13	1	1	ns
PC.35.3	C-White	C-Honey	5	5	12	1	1	ns

Abbreviations: Cer, Ceramide; CL, Cardiolipin; DGDG, Digalactosyldiacylglycerol

DGGA, Diacylglycerylglucuronide; DGTS, Diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGDG, Monogalactosyldiacylglycerol; LPC, Lysophosphatidylcholine; LPE, Lysophosphatidylethanolamine; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; PI, Phosphatidylinositol; PS, Phosphatidylserine; SQDG, Sulfoquinovosyldiacylglycerol. .*Statistically significant differences between mutants of *C. vulgaris* (*Wilcoxon rank sum test*, $q < 0.05$).

Table S4.6. Results of Wilcoxon rank-sum test (FDR adjusted) for statistically significant differences of lipid classes between C-Honey and C- White.

variable	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
DGGA	C-White	C-Honey	5	5	0	0,00794	0,0185267	*
LPE	C-White	C-Honey	5	5	0	0,00794	0,0185267	*
Cer	C-White	C-Honey	5	5	0	0,00794	0,0185267	*
DGDG	C-White	C-Honey	5	5	0	0,00794	0,0185267	*
LPC	C-White	C-Honey	5	5	0	0,00794	0,0185267	*
PS	C-White	C-Honey	5	5	25	0,00794	0,0185267	*

MGDG	C-White	C-Honey	5	5	25	0,0117	0,0234	*
PG	C-White	C-Honey	5	5	1	0,0159	0,0247333	*
DGTS	C-White	C-Honey	5	5	24	0,0159	0,0247333	*
CL	C-White	C-Honey	5	5	1	0,0212	0,02968	*
PI	C-White	C-Honey	5	5	3	0,0556	0,0707636	ns
PE	C-White	C-Honey	5	5	9,5	0,6	0,6	ns
SQDG	C-White	C-Honey	5	5	16	0,548	0,6	ns
PC	C-White	C-Honey	5	5	9,5	0,6	0,6	ns

Abbreviations: Cer, Ceramide; CL, Cardiolipin; DGDG, Digalactosyldiacylglycerol

DGGA, Diacylglycerylglucuronide; DGTS, Diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGDG, Monogalactosyldiacylglycerol; LPC, Lysophosphatidylcholine; LPE, Lysophosphatidylethanolamine; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; PI, Phosphatidylinositol; PS, Phosphatidylserine; SQDG, Sulfoquinovosyldiacylglycerol. *Statistically significant differences between mutants of *C. vulgaris* (*Wilcoxon rank sum test*, $q < 0.05$).

Supplementary material of Chapter 5

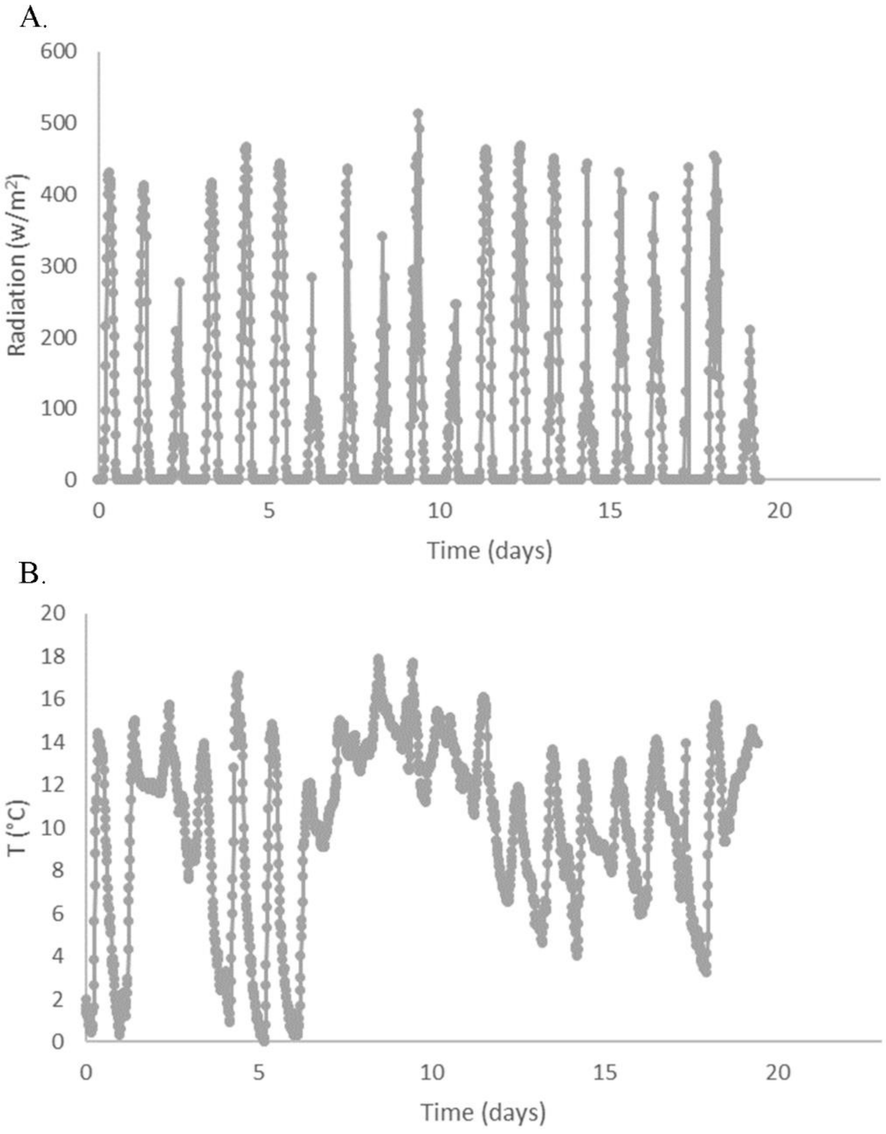
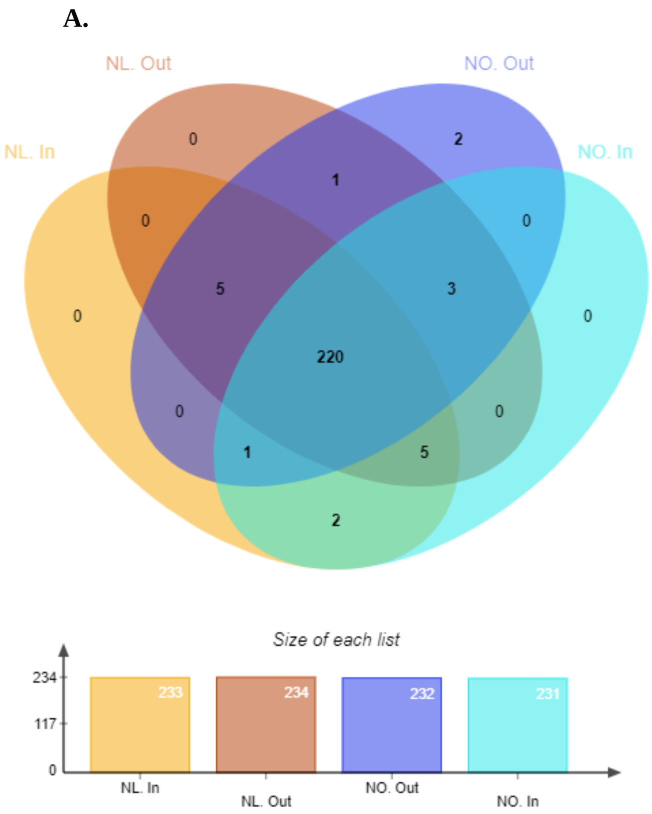


Figure S5.1. Graphical representation values of the (**A**) local solar radiation and (**B**) temperature in period January 7th to 27th of 2020 in Pataias, Portugal.



B.

Specific lipid species of the:
1. Indoor growth
MGDG(34:2)
MGDG(34:1)
2. Outdoor growth
PG(36:6)
3. NO grown under outdoor growth
PI(28:0)
DGTS(36:3)

Figure S5.2. (**A**) Venn diagram of polar lipid species identified in total lipid extracts of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In). (**B**) Specific polar lipid species for indoor and outdoor cultivation, and outdoor-grown NO.

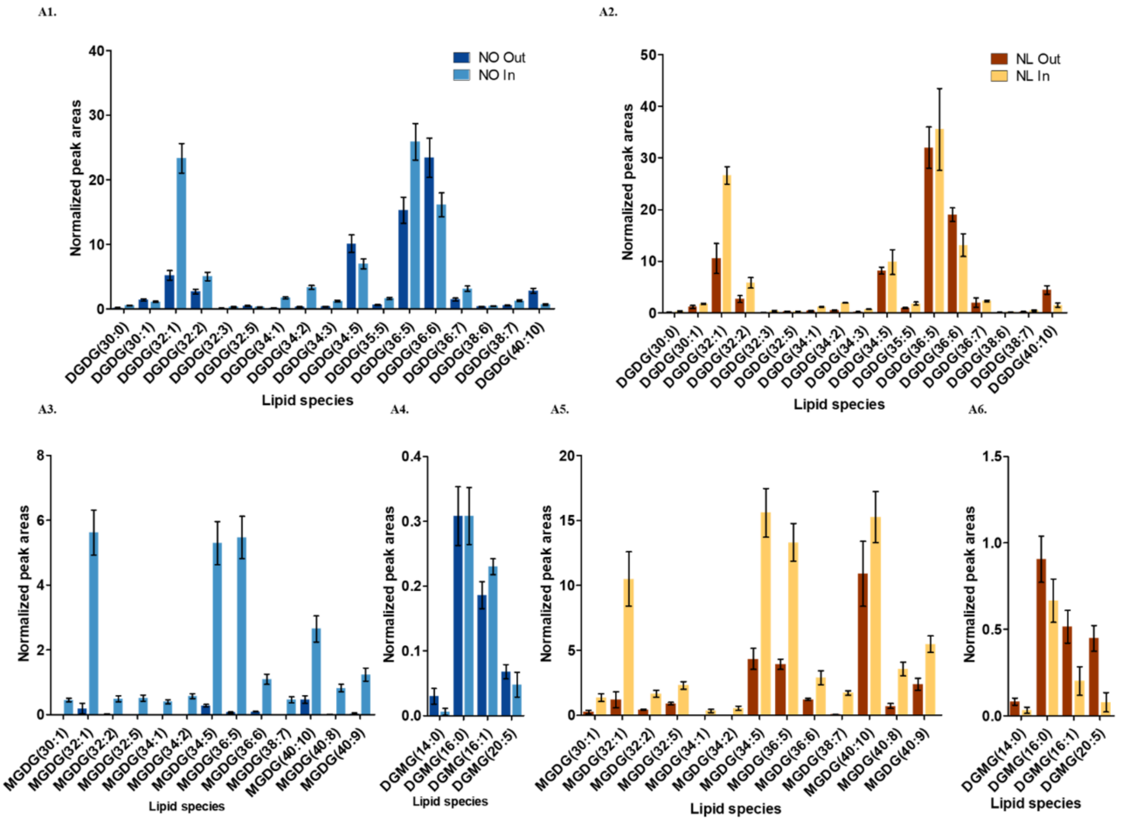


Figure S5.3. Glycolipid species identified in the total lipid extracts of *N. oceanica* (NO) (represented in blue) and *N. limnetica* (NL) (represented in brown) grown outdoors (Out) and indoors (In). (**A1;A2**) digalactosyldiacylglycerol (DGDG), (**A3; A5**) monogalactosyldiacylglycerol (MGDG); (**A4;A6**) digalactosylmonoacylglycerol (DGMG) lipid species. The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments.

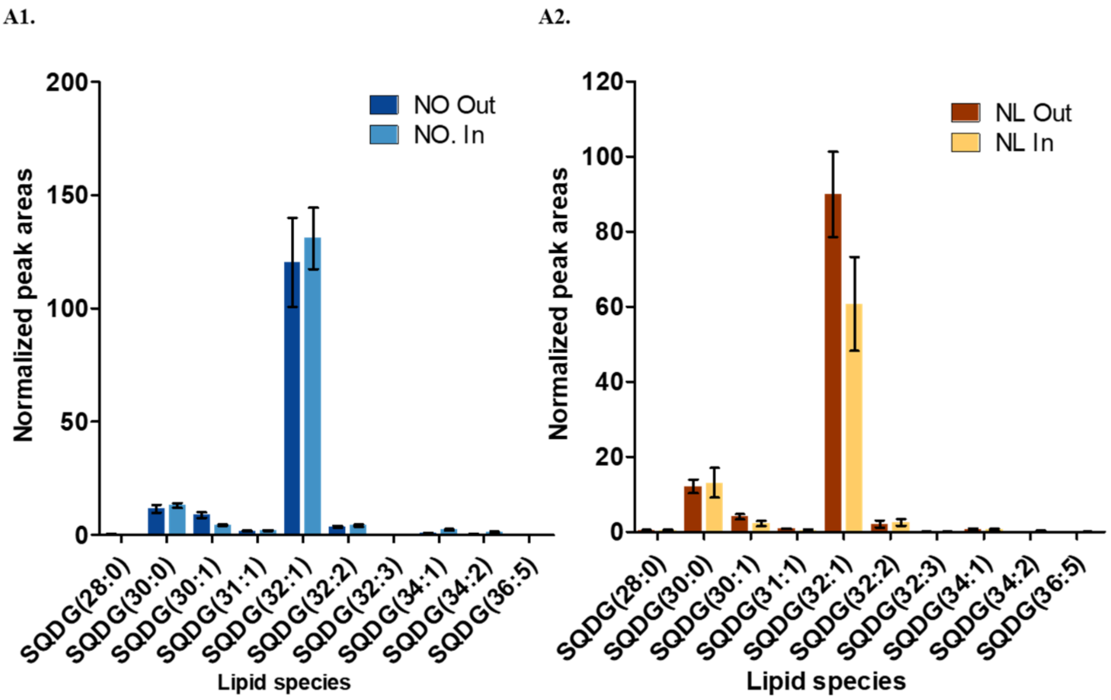


Figure S5.4. Glycolipid species identified in the total lipid extracts of *N. oceanica* (NO) (represented in blue) and *N. limnetica* (NL) (represented in brown) grown outdoors (Out) and indoors (In). (**A1;A2**) sulfoquinovosyldiacylglycerol (SQDG) lipid species. The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments.

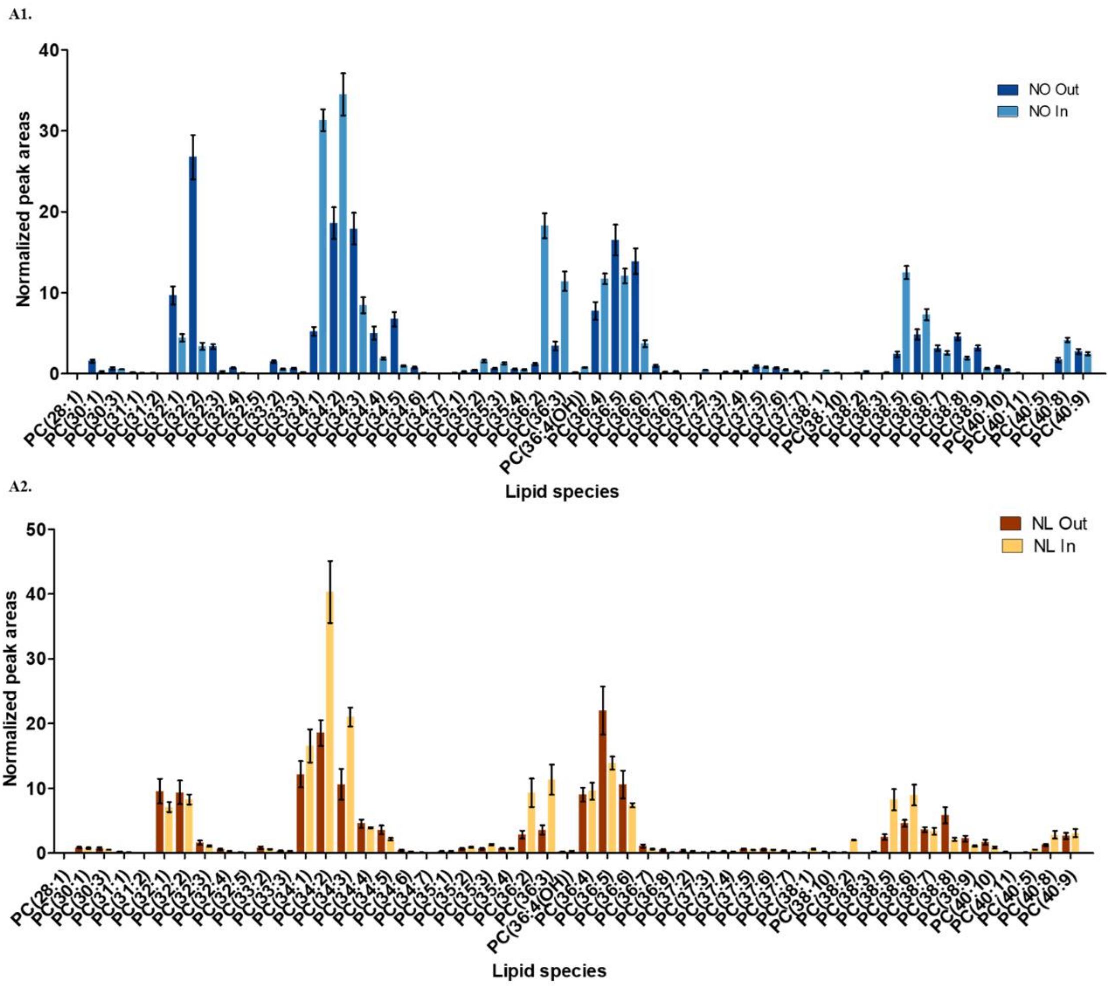


Figure S5.5. Phospholipid species identified in the total lipid extracts of *N. oceanica* (NO) (represented in blue) and *N. limnetica* (NL) (represented in brown) grown outdoors (Out) and indoors (In). (**A1;A2**) Phosphatidylcholine (PC) lipid species. The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments.

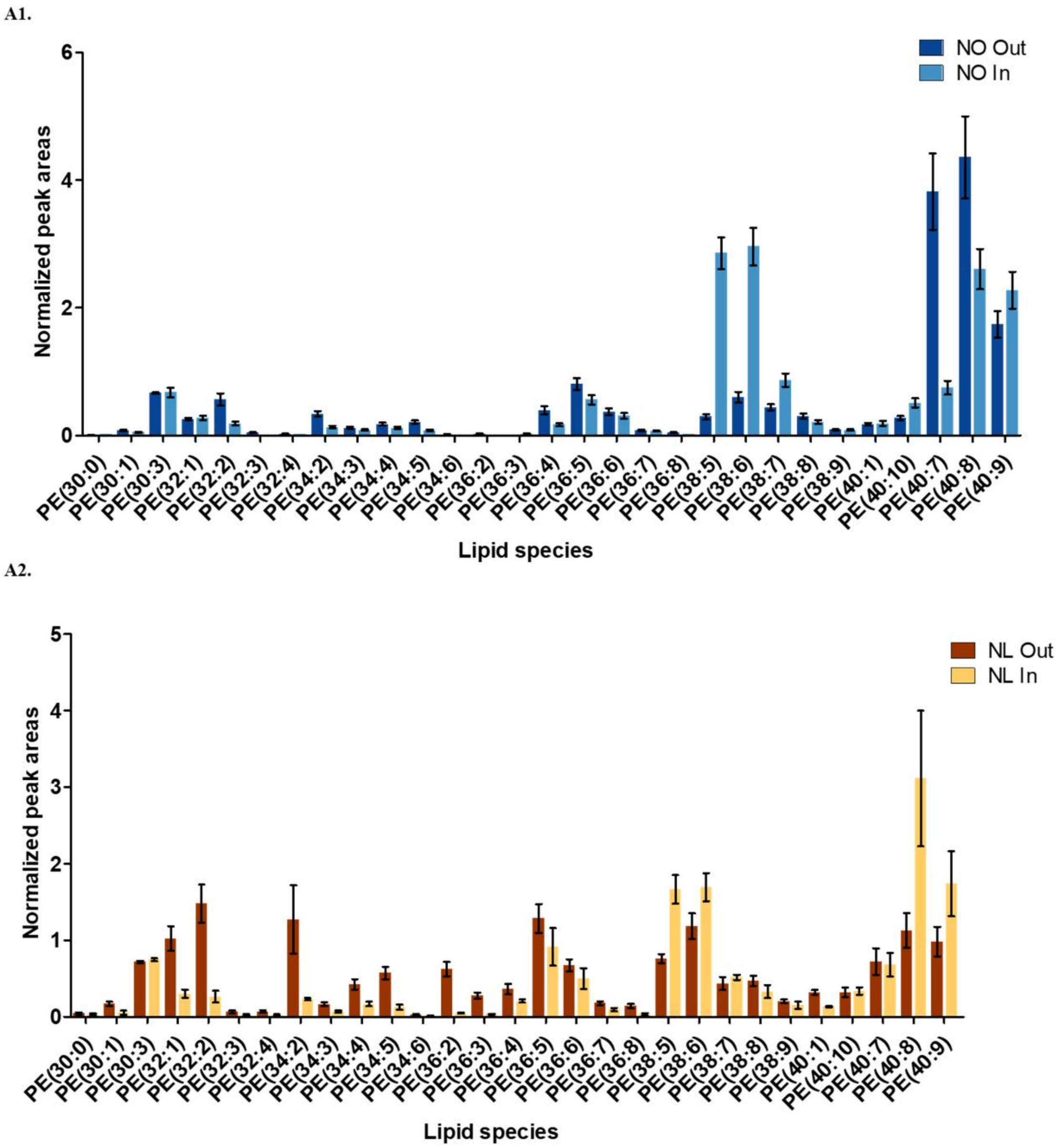


Figure S5.6. Phospholipid species identified in the total lipid extracts of *N. oceanica* (NO) (represented in blue) and *N. limnetica* (NL) (represented in brown) grown outdoors (Out) and indoors (In) conditions. (**A1;A2**) Phosphatidylethanolamine (PE) lipid species. The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments.

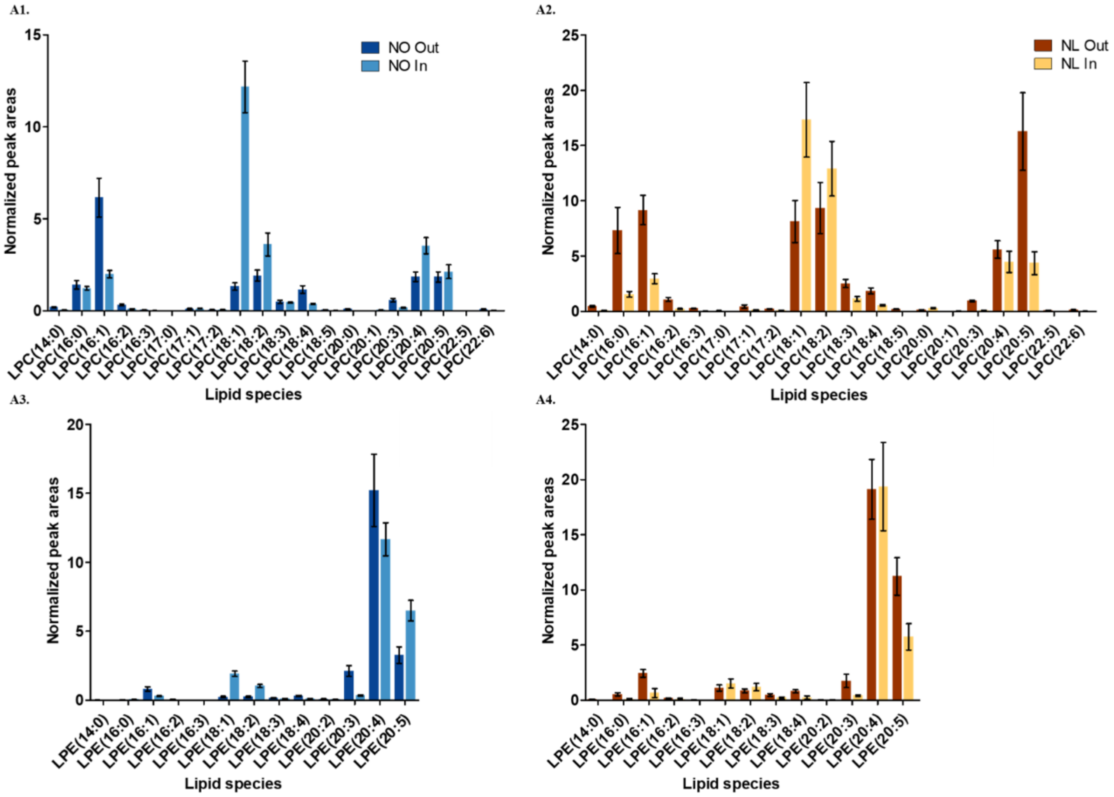


Figure S5.7. Lyso phospholipid species identified in the total lipid extracts of *N. oceanica* (NO) (represented in blue) and *N. limnetica* (NL) (represented in brown) grown outdoors (Out) and indoors (In). (**A1; A2**) Lysophosphatidylcholine (LPC), (**A3; A4**) lysophosphatidylethanolamine (LPE) lipid species. The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments.

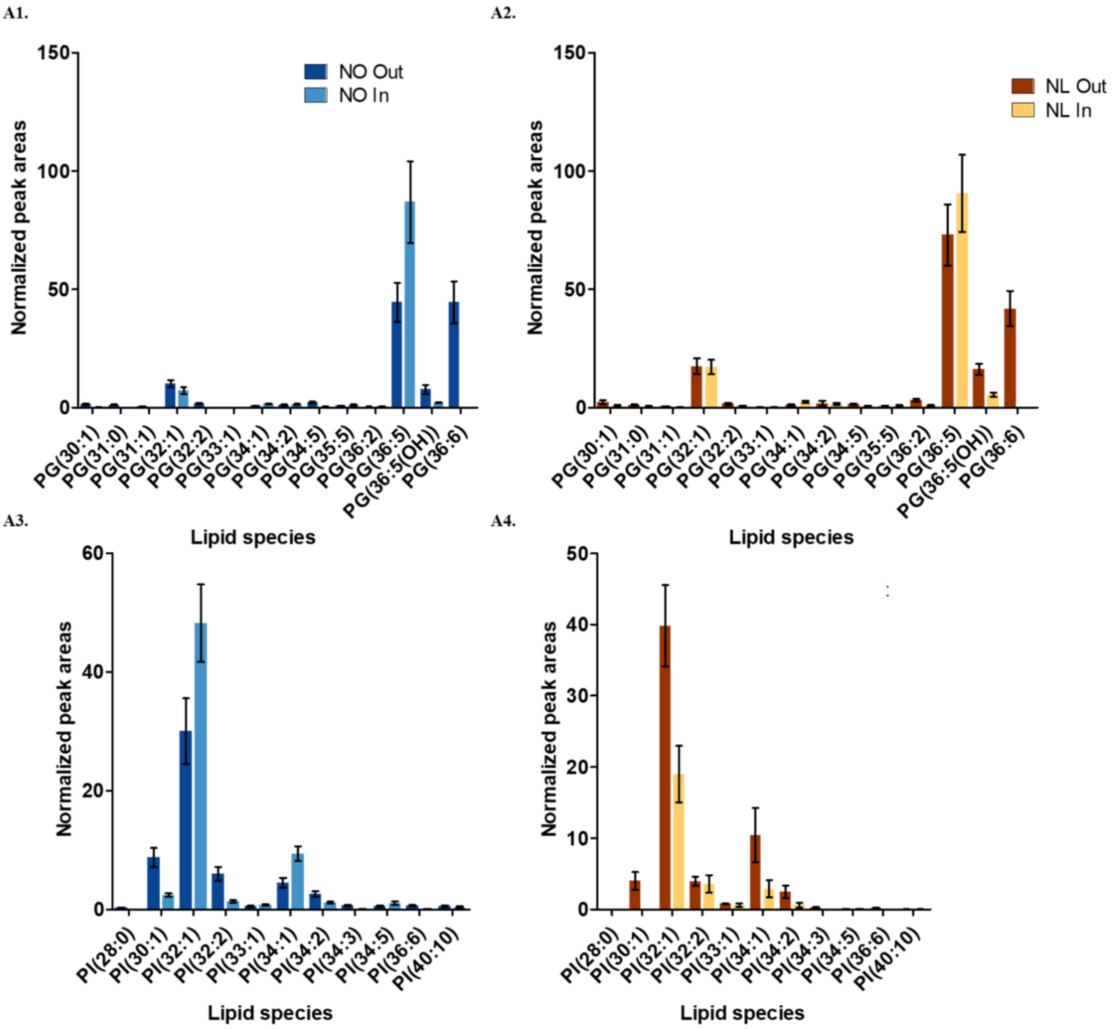


Figure S5.8. Phospholipid species identified in the total lipid extracts of *N. oceanica* (NO) (represented in blue) and *N. limnetica* (NL) (represented in brown) grown outdoors (Out) and indoors (In). (**A1; A2**) Phosphatidylglycerol (PG), (**A3; A4**) phosphatidylinositol (PI) lipid species. The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments.

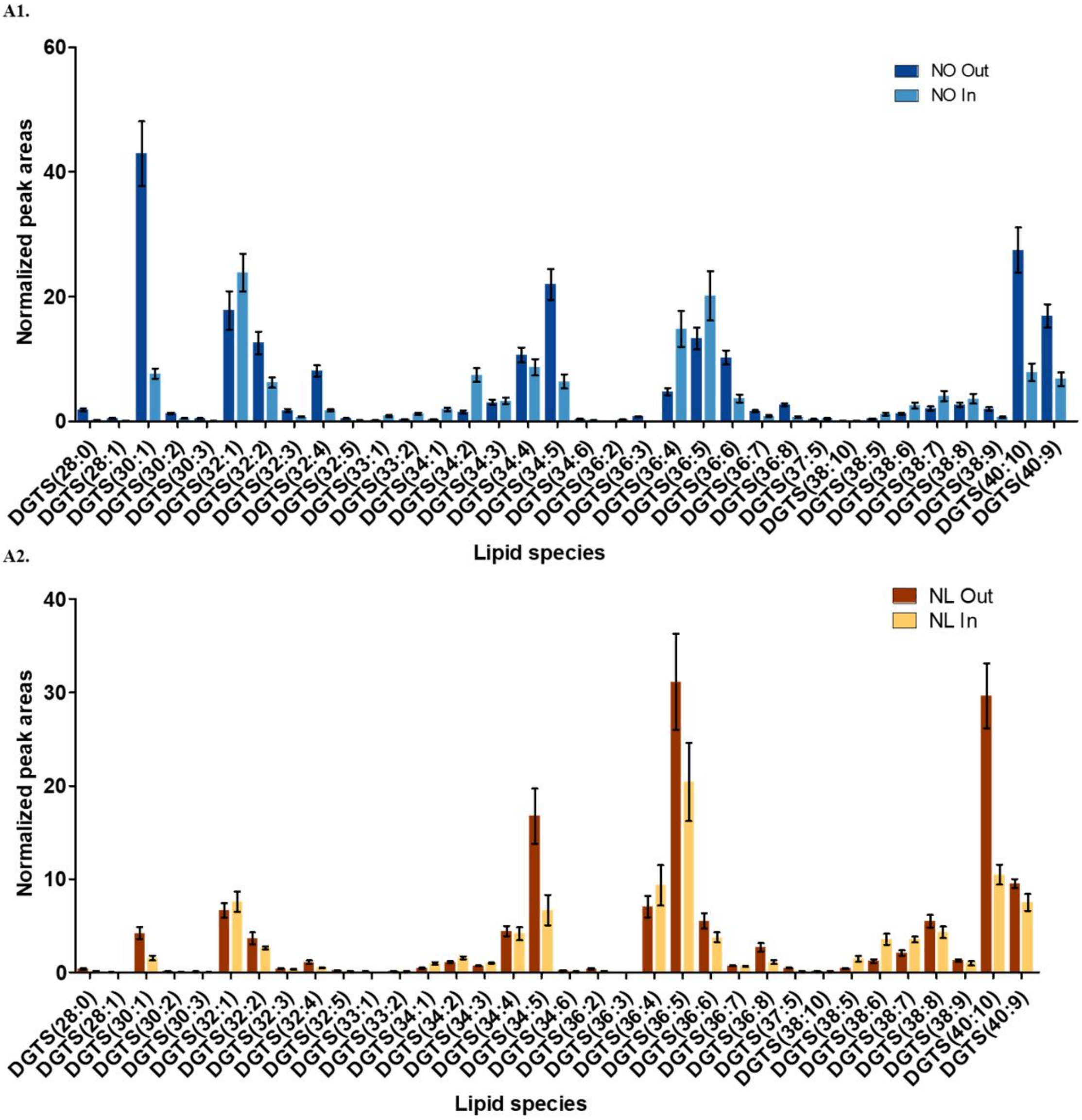


Figure S5.9. Betaine lipid species identified in the total lipid extracts of *N. oceanica* (NO) (represented in blue) and *N. limnetica* (NL) (represented in brown) grown outdoors (Out) and indoors (In). (**A1;A2**) diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine (DGTS) lipid species. The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments.

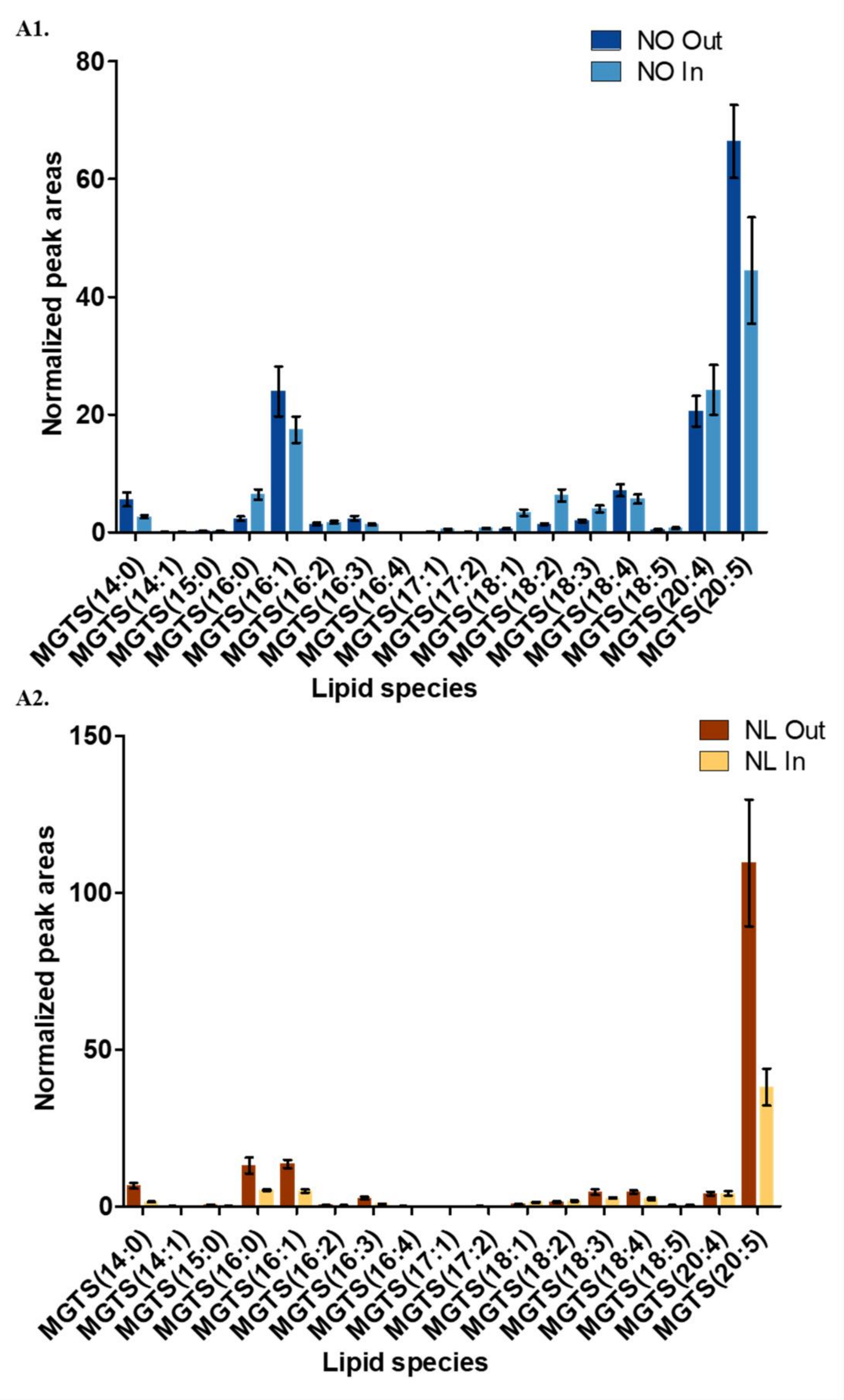


Figure S5.10. Lyso-betaine lipid species identified in the total lipid extracts of *N. oceanica* (NO) (represented in blue) and *N. limnetica* (NL) (represented in brown) grown outdoors (Out) and indoors (In). (**A1;A2**) Monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine (MGTS) lipid species. The numbers in parentheses (C: N) represent the number of carbon atoms (C) and double bonds (N) in the fatty acid side chains. Data are represented as the mean ± standard deviation of five independent experiments.

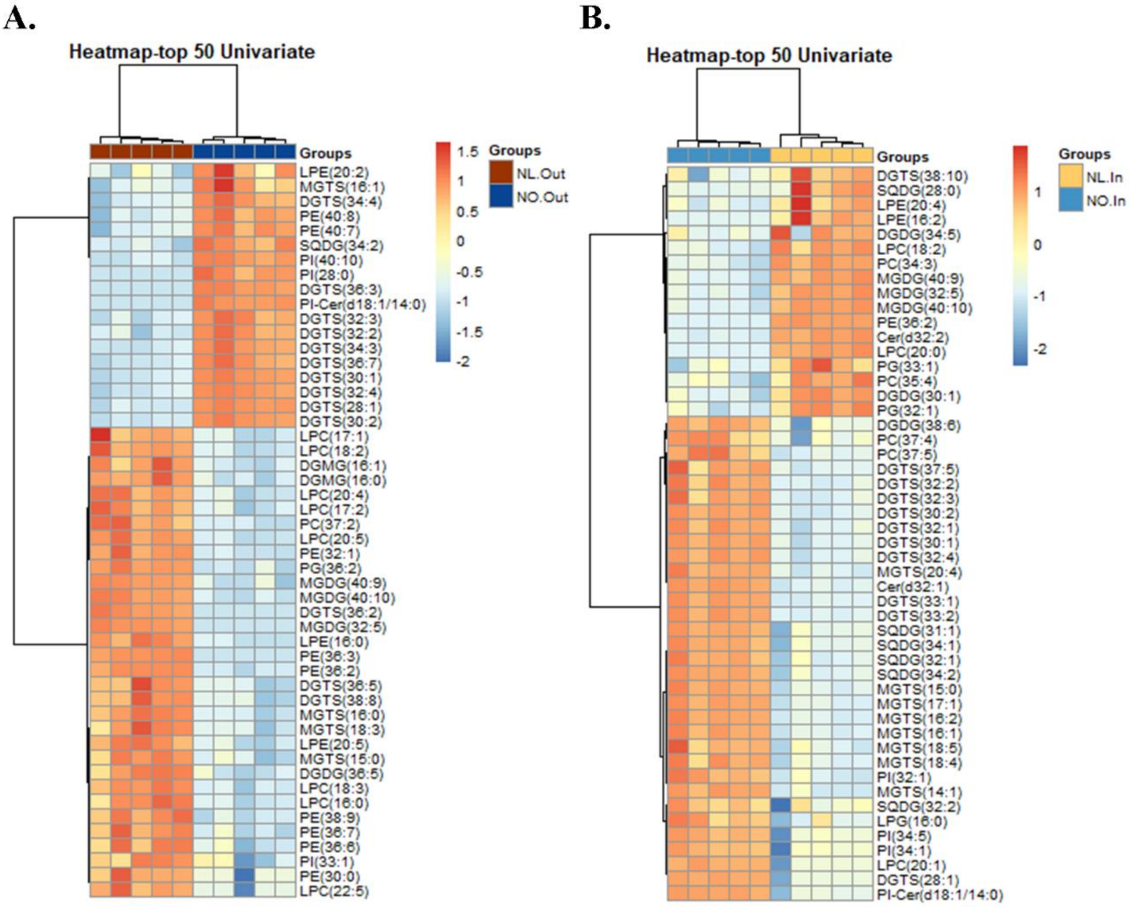


Figure S5.11. Two-dimensional hierarchical clustering heat map of the 50 most significant polar lipids between *N. oceanica* (NO) and *N. limnetica* (NL) grown (**A**) outdoors (Out) and (**B**) indoors (In) (lower *q* values). The relative abundance levels are indicated on the colour scale and the numbers indicate the fold difference from the mean. The dendrogram at the top represents the clustering of the sample groups, showing two main clusters, one for each group. The dendrogram on the left represents the clustering of individual lipid species given their similarity in relative abundance. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; and PI-Cer, inositolphosphoceramide.

Table S5.1. Results of one-way ANOVA test (FDR adjusted) for statistically significant differences of lipid content and the amount of phospholipids (PL), glycolipids (GL), and neutral lipids plus pigments in the total lipid extracts between the groups.

Variables	DFn	DFd	F	*p*	*p*<.05	ges	*p*.adj	*p*.adj.signif
GL Content	3	16	5.56	0.00800	*	0.51	0.01067	*
Lipid Content	3	16	12.43	0.00019	*	0.7	0.00038	***
Neutral lipids+ Pigments Content	3	16	2.77	0.07600		0.342	0.07600	ns
PL Content	3	16	38.63	0.00000	*	0.879	0.00000	****

Table S5.2. Results of Tukey multiple comparison post-hoc test (FDR adjusted) for statistically significant differences of lipid content and the amount of phospholipids (PL) and glycolipids (GL) in the total lipid extracts between the groups.

Variables	group1	group2	null.value	estimate	conf.low	conf.high	p.adj	p.adj.signif
GL Content	NL-In	NL-Out	0	-0.035	-0.092	0.0219	0.328	ns
GL Content	NL-In	NO-In	0	0.0363	-0.0206	0.0933	0.298	ns
GL Content	NL-In	NO-Out	0	-0.0314	-0.0884	0.0255	0.418	ns
GL Content	NL-Out	NO-In	0	0.0713	0.0144	0.1283	0.0119	*
GL Content	NL-Out	NO-Out	0	0.0036	-0.0533	0.0606	0.998	ns
GL Content	NO-In	NO-Out	0	-0.0677	-0.1247	-0.0108	0.0172	*
Lipid Content	NL-In	NL-Out	0	-0.0375	-0.126	0.0509	0.627	ns
Lipid Content	NL-In	NO-In	0	0.139	0.0505	0.2275	0.00188	**
Lipid Content	NL-In	NO-Out	0	0.0069	-0.0816	0.0954	0.996	ns
Lipid Content	NL-Out	NO-In	0	0.1765	0.088	0.265	0.000172	***
Lipid Content	NL-Out	NO-Out	0	0.0444	-0.0441	0.1329	0.496	ns
Lipid Content	NO-In	NO-Out	0	-0.1321	-0.2206	-0.0436	0.00295	**
PL Content	NL-In	NL-Out	0	0.21	0.135	0.2851	0.000003	****
PL Content	NL-In	NO-In	0	0.1196	0.0445	0.1947	0.00165	**
PL Content	NL-In	NO-Out	0	0.2636	0.1885	0.3387	0.0000001	****
PL Content	NL-Out	NO-In	0	-0.0904	-0.1655	-0.0153	0.0158	*
PL Content	NL-Out	NO-Out	0	0.0535	-0.0216	0.1286	0.215	ns
PL Content	NO-In	NO-Out	0	0.144	0.0689	0.219	0.000264	***

Table S5.3. Results of one-way ANOVA test (FDR adjusted) for statistically significant differences of content of phospholipids (PL), glycolipids (GL), and neutral lipids plus pigments in the biomass between the groups.

Variables	DFn	DFd	F	p	p<.05	ges	p.adj	p.adj.signif
GL Content	3	16	16.276	0.0000406	*	0.753	0.0001034	***
Neutral lipids + Pigments Content	3	16	10.014	0.00059	*	0.652	0.00059	***
PL Content	3	16	14.871	0.0000689	*	0.736	0.0001034	***

Table S5.4. Results of Tukey multiple comparison post-hoc test (FDR adjusted) for statistically significant differences of content of phospholipids (PL), glycolipids (GL), and neutral lipids plus pigments in the biomass between the groups.

Variables	group1	group2	null.value	estimate	conf.low	conf.high	p.adj	p.adj.signif
GL.Content	NL-In	NL-Out	0	-0.0727	-0.1813	0.0359	0.261	ns
GL Content	NL-In	NO-In	0	0.1756	0.067	0.2842	0.00144	**
GL Content	NL-In	NO-Out	0	-0.0245	-0.1331	0.0841	0.916	ns
GL Content	NL-Out	NO-In	0	0.2483	0.1397	0.3569	0.0000367	****
GL Content	NL-Out	NO-Out	0	0.0482	-0.0604	0.1568	0.594	ns
GL Content	NO-In	NO-Out	0	-0.2001	-0.3087	-0.0915	0.000399	***
Neutral lipids + Pigments Content	NL-In	NL-Out	0	-0.0466	-0.1358	0.0427	0.464	ns
Neutral lipids + Pigments Content	NL-In	NO-In	0	0.1146	0.0254	0.2039	0.00994	**
Neutral lipids + Pigments Content	NL-In	NO-Out	0	-0.0111	-0.1003	0.0781	0.984	ns
Neutral lipids + Pigments Content	NL-Out	NO-In	0	0.1612	0.0719	0.2504	0.000491	***
Neutral lipids + Pigments Content	NL-Out	NO-Out	0	0.0355	-0.0538	0.1247	0.673	ns
Neutral lipids + Pigments Content	NO-In	NO-Out	0	-0.1257	-0.215	-0.0365	0.00482	**
PL Content	NL-In	NL-Out	0	0.1721	0.0413	0.3029	0.00829	**
PL Content	NL-In	NO-In	0	0.2585	0.1277	0.3893	0.000191	***

PL Content	NL-In	NO-Out	0	0.2701	0.1393	0.4009	0.000118	***
PL Content	NL-Out	NO-In	0	0.0864	-0.0444	0.2172	0.271	ns
PL Content	NL-Out	NO-Out	0	0.098	-0.0328	0.2288	0.182	ns
PL Content	NO-In	NO-Out	0	0.0116	-0.1192	0.1424	0.994	ns

Table S5.5. GC-MS analysis of the fatty acid composition expressed as mg FA/100 mg of lipid extracts of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In). Data are means ± SD, n=5.

	Mean ± STD				***q* values**	
Fatty acid	**NO.Out**	**NO.In**	**NL.Out**	**NL.In**	**NO.Out vs NO.In**	**NL.Out vs NL.In**
C12:0	1.3±0.2	1.2±0.3	1.2±0.1	0.9±0.2	ns	ns
C14:0	5.6±0.8	7.2±2.2	3.9±0.8	4.7±0.3	ns	ns
C15:0	1.3±0.2	1.4±0.2	1.3±0.1	1.0±0.2	ns	ns
C16:0	**10.6±1.4**	**30.9±3.1**	**12.6±2.0**	**22.7±2.0**	**	ns
C16:1 Δ^9 (*n*-7)	**18.8±2.5**	**20.3±2.4**	**13.0±3.2**	**16.5±1.6**	ns	ns
C16:1 Δ^7 (*n*-9)	1.8±0.2	1.4±0.1	1.7±0.1	1.1±0.3	ns	*
C16:2 $\Delta^{7,10}$ (*n*-6)	1.7±0.2	1.3±0.1	1.6±0.1	1.2±0.3	ns	ns
C17:0	2.1±0.3	1.7±0.1	2.0±0.1	1.5±0.3	ns	ns
C17:1 Δ^8 (*n*-9)	1.7±0.2	1.5±0.1	1.6±0.1	1.2±0.2	ns	ns
C18:0	4.8±0.7	3.9±0.3	4.6±0.2	3.6±0.9	ns	ns
C18:1 Δ^9 (*n*-9)	3.6±0.4	**11.6±0.7**	3.7±0.7	**10.6±1.8**	*	ns
C18:1 Δ^{11} (*n*-7)	1.7±0.2	1.5±0.1	1.9±0.1	1.3±0.3	ns	*

C18:2 $\Delta^{9,12}$ (*n*-6)	4.3±0.5	4.1±0.1	3.6±0.3	3.8±0.4	ns	ns
C18:3 (*n*-6)	3.1±0.4	2.4±0.2	2.9±0.2	2.0±0.5	ns	*
C20:3 (*n*-6)	3.5±0.5	2.6±0.2	3.2±0.2	2.3±0.5	*	ns
C20:4 (*n*-6)	**6.7±0.9**	4.3±0.2	4.5±0.4	4.5±0.4	*	ns
C20:5 (*n*-3)	**14.3±1.9**	**10.5±0.9**	**13.3±1.3**	**9.4±0.8**	ns	*
∑ **PUFA**	**33.7±4.3**	25.1±0.9	**29.0±2.4**	23.1±2.6	*	ns
∑ (*n*-3)	14.3±1.9	10.5±0.9	13.3±1.3	9.4±0.8	ns	*
∑ (*n*-6)	19.4±2.5	14.6±0.5	15.8±1.2	13.7±1.8	ns	ns
∑ MUFA	27.6±3.5	36.3±2.9	21.9±4.0	30.7±3.2	ns	ns
∑ SFA	25.7±3.3	**46.4±5.8**	25.5±3.2	34.4±3.0	*	ns
n-6/*n*-3 ratio	1.4±0.1	1.4±0.1	1.2±0.0	1.4±0.1	ns	*
h/H ratio	**2.2±0.0**	1.0±0.1	**2.0±0.2**	1.2±0.1	**	ns
AI	**0.6±0.0**	1.0±0.2	**0.6±0.0**	0.8±0.0	**	ns
TI	**0.3±0.0**	0.7±0.1	**0.4±0.0**	0.6±0.0	**	ns

The most abundant FA are highlighted in bold font. NO, *N. oceanica*; NL, *N. limnetica*; Out, grown outdoors; In, grown indoors; ∑ PUFA, sum of polyunsaturated fatty acids; ∑ MUFA, sum of mono-unsaturated fatty acids; ∑ SFA, sum of saturated fatty acids; h/H ratio, hypocholesterolemic/hypercholesterolemic indices; AI, atherogenic index; TI, thrombogenic index; SD, standard deviation; ns, $q > 0.05$; *, $q < 0.05$; **, $q < 0.01$; ***, $q < 0.001$; ****, $q < 0.0001$.

Table S5.6. Exact mass measurements of the molecular ions identified by HILIC−ESI−MS in the total lipid extracts of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In). C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains.

Lipid species (C:N)	**Formula**	**Calculated m/z**	**Observed *m/z* in MS of the NO Out extracts**	**Error (ppm)**	**Observed *m/z* in MS of the NO In extracts**	**Error (ppm)**	**Observed *m/z* in MS of the NL Out extracts**	**Error (ppm)**	**Observed *m/z* in MS of the NL In extracts**	**Error (ppm)**

LPC identified as $[M+H]^+$										
LPC(14:0)	C22H47NO7P	468.309	468.3081	**-1.96**	468.3085	**-1.1**	468.3088	**-0.46**	468.3087	**-0.68**
LPC(16:3)	C24H45NO7P	490.2934	490.2913	**-4.22**	490.2909	**-5.03**	490.2921	**-2.58**	490.2922	**-2.38**
LPC(16:2)	C24H47NO7P	492.309	492.3081	**-1.86**	492.3074	**-3.28**	492.3091	**0.17**	492.3078	**-2.47**
LPC(16:1)	C24H49NO7P	494.3247	494.3239	**-1.55**	494.3243	**-0.74**	494.3246	**-0.14**	494.3244	**-0.54**
LPC(16:0)	C24H51NO7P	496.3403	496.3401	**-0.44**	496.3401	**-0.44**	496.34	**-0.64**	496.3402	**-0.23**
LPC(17:0)	C25H53NO7P	510.356	510.3561	**0.26**	510.3562	**0.46**	510.3565	**1.05**	510.3559	**-0.13**
LPC(17:1)	C25H51NO7P	508.3403	508.3398	**-1.02**	508.3403	**-0.03**	508.3402	**-0.23**	508.3401	**-0.42**
LPC(17:2)	C25H49NO7P	506.3247	506.3238	**-1.71**	506.3244	**-0.53**	506.3243	**-0.72**	506.3239	**-1.51**
LPC(18:5)	C26H45NO7P	514.2934	514.291	**-4.6**	514.2911	**-4.41**	514.2911	**-4.41**	514.291	**-4.6**
LPC(18:4)	C26H47NO7P	516.309	516.3065	**-4.87**	516.3066	**-4.68**	516.3068	**-4.29**	516.3065	**-4.87**
LPC(18:3)	C26H49NO7P	518.3247	518.3232	**-2.83**	518.3238	**-1.67**	518.3231	**-3.02**	518.3241	**-1.09**
LPC(18:2)	C26H51NO7P	520.3403	520.3398	**-0.99**	520.3402	**-0.22**	520.3403	**-0.03**	520.3403	**-0.03**
LPC(18:1)	C26H53NO7P	522.356	522.3555	**-0.89**	522.3558	**-0.32**	522.356	**0.06**	522.3558	**-0.32**
LPC(20:5)	C28H49NO7P	542.3247	542.3234	**-2.34**	542.3238	**-1.6**	542.3244	**-0.49**	542.323	**-3.07**
LPC(20:4)	C28H51NO7P	544.3403	544.3395	**-1.5**	544.3386	**-3.15**	544.3395	**-1.5**	544.3384	**-3.52**
LPC(20:3)	C28H53NO7P	546.356	546.3556	**-0.67**	546.3562	**0.43**	546.3562	**0.43**	546.3546	**-2.5**
LPC(20:1)	C28H57NO7P	550.3873	550.3865	**-1.39**	550.3872	**-0.12**	550.3874	**0.24**	550.3875	**0.42**
LPC(20:0)	C28H59NO7P	552.4029	552.402	**-1.66**	552.4023	**-1.12**	552.4023	**-1.12**	552.4026	**-0.57**
LPC(22:6)	C30H51NO7P	568.3403	568.3375	**-4.95**	568.3379	**-4.25**	568.338	**-4.08**	568.3375	**-4.95**
LPC(22:5)	C30H53NO7P	570.356	570.3542	**-3.1**	570.3547	**-2.22**	570.3559	**-0.12**	570.355	**-1.7**
PC identified as $[M+H]^+$										
PC(28:1)	C36H71NO8P	676.4917	676.4907	**-1.53**	676.4911	**-0.93**	676.491	**-1.08**	676.4911	**-0.93**
PC(30:3)	C38H71NO8P	700.4917	700.4886	**-4.47**	700.4893	**-3.47**	700.4894	**-3.33**	700.4893	**-3.47**
PC(30:1)	C38H75NO8P	704.523	704.5225	**-0.76**	704.5242	**1.66**	704.5233	**0.38**	704.5236	**0.81**
PC(31:2)	C39H75NO8P	716.523	716.5226	**-0.6**	716.5214	**-2.28**	716.5226	**-0.6**	716.5225	**-0.74**
PC(31:1)	C39H77NO8P	718.5387	718.5378	**-1.23**	718.538	**-0.95**	718.5382	**-0.67**	718.5377	**-1.37**
PC(32:5)	C40H71NO8P	724.4917	724.4897	**-2.8**	724.4907	**-1.42**	724.4906	**-1.56**	724.4903	**-1.98**
PC(32:4)	C40H73NO8P	726.5074	726.5055	**-2.59**	726.505	**-3.28**	726.5061	**-1.76**	726.5059	**-2.04**

PC(32:3)	C40H75NO8P	728.523	728.5225	**-0.73**	728.5226	**-0.59**	728.5227	**-0.46**	728.5227	**-0.46**
PC(32:2)	C40H77NO8P	730.5387	730.5382	**-0.66**	730.5384	**-0.39**	730.5387	**0.02**	730.5385	**-0.25**
PC(32:1)	C40H79NO8P	732.5543	732.5516	**-3.73**	732.5539	**-0.59**	732.5536	**-1**	732.5534	**-1.27**
PC(33:3)	C41H77NO8P	742.5387	742.5378	**-1.19**	742.5381	**-0.78**	742.5382	**-0.65**	742.5383	**-0.51**
PC(33:2)	C41H79NO8P	744.5543	744.5532	**-1.52**	744.5539	**-0.58**	744.554	**-0.44**	744.5541	**-0.31**
PC(34:7)	C42H71NO8P	748.4917	748.488	**-4.99**	_	**_**	748.4892	**-3.38**	748.4887	**-4.05**
PC(34:6)	C42H73NO8P	750.5074	750.5042	**-4.24**	750.5052	**-2.91**	750.5051	**-3.04**	750.5054	**-2.64**
PC(34:5)	C42H75NO8P	752.523	752.52	**-4.03**	752.5208	**-2.97**	752.5217	**-1.77**	752.521	**-2.7**
PC(34:4)	C42H77NO8P	754.5387	754.5356	**-4.08**	754.5365	**-2.89**	754.5369	**-2.36**	754.5375	**-1.57**
PC(34:3)	C42H79NO8P	756.5543	756.5536	**-0.97**	756.5541	**-0.31**	756.5538	**-0.7**	756.554	**-0.44**
PC(34:2)	C42H81NO8P	758.57	758.5683	**-2.22**	758.5698	**-0.24**	758.5694	**-0.77**	758.5693	**-0.9**
PC(34:1)	C42H83NO8P	760.5856	760.5821	**-4.64**	760.5846	**-1.36**	760.5844	**-1.62**	760.5835	**-2.8**
PC(35:4)	C43H79NO8P	768.5543	768.5514	**-3.81**	768.5517	**-3.42**	768.5521	**-2.9**	768.5532	**-1.47**
PC(35:3)	C43H81NO8P	770.57	770.5682	**-2.31**	770.5691	**-1.14**	770.5686	**-1.79**	770.5691	**-1.14**
PC(35:2)	C43H83NO8P	772.5856	772.5843	**-1.72**	772.5846	**-1.34**	772.585	**-0.82**	772.5842	**-1.85**
PC(35:1)	C43H85NO8P	774.6013	774.5986	**-3.46**	774.5993	**-2.56**	774.6	**-1.66**	774.5981	**-4.11**
PC(37:7)	C45H77NO8P	790.5387	790.535	**-4.66**	790.5366	**-2.63**	790.535	**-4.66**	790.5351	**-4.53**
PC(37:6)	C45H79NO8P	792.5543	792.5522	**-2.69**	792.5521	**-2.81**	792.5521	**-2.81**	792.5517	**-3.32**
PC(36:8)	C44H73NO8P	774.5074	774.5072	**-0.23**	774.5039	**-4.5**	774.5052	**-2.82**	774.5055	**-2.43**
PC(36:7)	C44H75NO8P	776.523	776.52	**-3.9**	776.5192	**-4.93**	776.5213	**-2.23**	776.5206	**-3.13**
PC(36:6)	C44H77NO8P	778.5387	778.5373	**-1.78**	778.5371	**-2.03**	778.5378	**-1.13**	778.5369	**-2.29**
PC(36:5)	C44H79NO8P	780.5543	780.5522	**-2.73**	780.5519	**-3.11**	780.5537	**-0.81**	780.5517	**-3.37**
PC(36:4)	C44H81NO8P	782.57	782.5672	**-3.55**	782.5671	**-3.68**	782.5665	**-4.45**	782.5675	**-3.17**
PC(36:4(OH))	C44H81NO9P	798.5649	798.5631	**-2.25**	798.5652	**0.38**	798.5648	**-0.12**	798.5633	**-2**
PC(36:3)	C44H83NO8P	784.5856	784.5836	**-2.59**	784.5847	**-1.19**	784.5833	**-2.97**	784.5845	**-1.44**
PC(36:2)	C44H85NO8P	786.6013	786.5992	**-2.65**	786.6004	**-1.12**	786.6005	**-0.99**	786.5999	**-1.76**
PC(37:5)	C45H81NO8P	794.57	794.5679	**-2.62**	794.5673	**-3.38**	794.5676	**-3**	794.567	**-3.75**
PC(37:4)	C45H83NO8P	796.5856	796.5825	**-3.93**	796.5826	**-3.81**	796.583	**-3.3**	796.5827	**-3.68**
PC(37:3)	C45H85NO8P	798.6013	798.5975	**-4.74**	798.5986	**-3.36**	798.5982	**-3.86**	798.5999	**-1.73**
PC(37:2)	C45H87NO8P	800.6169	800.6151	**-2.29**	800.616	**-1.16**	800.6168	**-0.16**	800.616	**-1.16**

PC(38:10)	C46H73NO8P	798.5074	798.5035	**-4.86**	798.505	**-2.98**	798.5038	**-4.49**	798.5039	**-4.36**
PC(38:9)	C46H75NO8P	800.523	800.5197	**-4.16**	800.5206	**-3.04**	800.5203	**-3.41**	800.5213	**-2.16**
PC(38:8)	C46H77NO8P	802.5387	802.5349	**-4.71**	802.5358	**-3.59**	802.5356	**-3.84**	802.5355	**-3.96**
PC(38:7)	C46H79NO8P	804.5543	804.5507	**-4.51**	804.5523	**-2.52**	804.5513	**-3.77**	804.5524	**-2.4**
PC(38:6)	C46H81NO8P	806.57	806.5682	**-2.21**	806.5682	**-2.21**	806.5687	**-1.59**	806.5687	**-1.59**
PC(38:5)	C46H83NO8P	808.5856	808.5829	**-3.38**	808.584	**-2.02**	808.583	**-3.26**	808.5837	**-2.39**
PC(38:3)	C46H87NO8P	812.6169	812.6139	**-3.73**	812.6149	**-2.5**	812.6165	**-0.53**	812.6176	**0.82**
PC(38:2)	C46H89NO8P	814.6326	814.6301	**-3.05**	814.6315	**-1.33**	814.6305	**-2.56**	814.6299	**-3.29**
PC(38:1)	C46H91NO8P	816.6482	816.6456	**-3.22**	816.6472	**-1.26**	816.6464	**-2.24**	816.6466	**-2**
PC(40:11)	C48H75NO8P	824.523	824.5199	**-3.8**	824.5211	**-2.34**	824.5204	**-3.19**	824.5209	**-2.59**
PC(40:10)	C48H77NO8P	826.5387	826.5352	**-4.21**	826.5363	**-2.88**	826.5371	**-1.91**	826.5356	**-3.73**
PC(40:9)	C48H79NO8P	828.5543	828.5516	**-3.3**	828.5514	**-3.54**	828.5518	**-3.05**	828.5512	**-3.78**
PC(40:8)	C48H81NO8P	830.57	830.5663	**-4.43**	830.5667	**-3.95**	830.5666	**-4.07**	830.5663	**-4.43**
PC(40:5)	C48H87NO8P	836.6169	836.6164	**-0.64**	836.6154	**-1.83**	836.6167	**-0.28**	836.6161	**-0.99**
LPE identified as $[M+H]^+$										
LPE(14:0)	C19H41NO7P	426.2621	426.2613	**-1.8**	426.2618	**-0.63**	426.2616	**-1.1**	426.2618	**-0.63**
LPE(16:3)	C21H39NO7P	448.2464	448.2442	**-4.94**	448.244	**-5.39**	448.2442	**-4.94**	448.2442	**-4.94**
LPE(16:2)	C21H41NO7P	450.2621	450.2612	**-1.93**	450.2613	**-1.7**	450.2617	**-0.82**	450.2615	**-1.26**
LPE(16:1)	C21H43NO7P	452.2777	452.2771	**-1.36**	452.2773	**-0.92**	452.2773	**-0.92**	452.2772	**-1.14**
LPE(16:0)	C21H45NO7P	454.2934	454.2945	**2.49**	454.2936	**0.51**	454.2934	**0.07**	454.294	**1.39**
LPE(18:4)	C23H41NO7P	474.2621	474.2597	**-4.99**	474.2599	**-4.57**	474.2597	**-4.99**	474.2597	**-4.99**
LPE(18:3)	C23H43NO7P	476.2777	476.2775	**-0.46**	476.2774	**-0.67**	476.2767	**-2.14**	476.2772	**-1.09**
LPE(18:2)	C23H45NO7P	478.2934	478.2929	**-0.98**	478.2931	**-0.56**	478.2932	**-0.35**	478.293	**-0.77**
LPE(18:1)	C23H47NO7P	480.309	480.309	**-0.04**	480.309	**-0.04**	480.309	**-0.04**	480.309	**-0.04**
LPE(20:5)	C25H43NO7P	500.2777	500.2772	**-1.03**	500.2774	**-0.63**	500.2775	**-0.43**	500.2773	**-0.83**
LPE(20:4)	C25H45NO7P	502.2934	502.2928	**-1.13**	502.2932	**-0.33**	502.2931	**-0.53**	502.2931	**-0.53**
LPE(20:3)	C25H47NO7P	504.309	504.3088	**-0.43**	504.3093	**0.56**	504.3089	**-0.23**	504.3081	**-1.82**
LPE(20:2)	C25H49NO7P	506.3247	506.3243	**-0.72**	506.3261	**2.83**	506.3244	**-0.53**	506.3238	**-1.71**
PE identified as $[M+H]^+$										

PE(30:3)	C35H65NO8P	658.4448	658.4417	**-4.68**	658.4422	**-3.92**	658.4424	**-3.62**	658.4421	**-4.07**
PE(30:1)	C35H69NO8P	662.4761	662.4757	**-0.58**	662.4794	**5.01**	662.4763	**0.33**	662.4764	**0.48**
PE(30:0)	C35H71NO8P	664.4917	664.4923	**0.85**	664.492	**0.4**	664.4921	**0.55**	664.4919	**0.25**
PE(32:4)	C37H67NO8P	684.4604	684.4588	**-2.38**	684.4621	**2.44**	684.4589	**-2.24**	684.4574	**-4.43**
PE(32:3)	C37H69NO8P	686.4761	686.4768	**1.05**	686.4785	**3.52**	686.4756	**-0.7**	686.4772	**1.63**
PE(32:2)	C37H71O8NP	688.4917	688.4912	**-0.77**	688.4919	**0.24**	688.4917	**-0.05**	688.4919	**0.24**
PE(32:1)	C37H73NO8P	690.5074	690.5064	**-1.42**	690.5073	**-0.12**	690.507	**-0.55**	690.5073	**-0.12**
PE(34:6)	C39H67NO8P	708.4604	708.4591	**-1.88**	708.4572	**-4.56**	708.4587	**-2.44**	708.4574	**-4.28**
PE(34:5)	C39H69NO8P	710.4761	710.4741	**-2.79**	710.4752	**-1.24**	710.4742	**-2.65**	710.4744	**-2.37**
PE(34:4)	C39H71NO8P	712.4917	712.4904	**-1.87**	712.4906	**-1.59**	712.49	**-2.43**	712.4898	**-2.71**
PE(34:3)	C39H73O8NP	714.5074	714.5069	**-0.67**	714.5066	**-1.09**	714.5069	**-0.67**	714.5067	**-0.95**
PE(34:2)	C39H75NO8P	716.523	716.521	**-2.84**	716.5207	**-3.25**	716.5224	**-0.88**	716.5226	**-0.6**
PE(36:8)	C41H67NO8P	732.4604	732.457	**-4.69**	732.4574	**-4.14**	732.4568	**-4.96**	732.4569	**-4.82**
PE(36:7)	C41H69NO8P	734.4761	734.4752	**-1.2**	734.4761	**0.02**	734.4739	**-2.97**	734.4735	**-3.52**
PE(36:6)	C41H71NO8P	736.4917	736.4907	**-1.4**	736.4918	**0.09**	736.4915	**-0.32**	736.4915	**-0.32**
PE(36:5)	C41H73O8NP	738.5074	738.5061	**-1.74**	738.5071	**-0.38**	738.5061	**-1.74**	738.5073	**-0.11**
PE(36:4)	C41H75NO8P	740.523	740.5234	**0.5**	740.5247	**2.25**	740.5226	**-0.58**	740.5194	**-4.9**
PE(36:3)	C41H77NO8P	742.5387	_	_	742.535	**-4.96**	742.5381	**-0.78**	742.5354	**-4.42**
PE(36:2)	C41H79O8NP	744.5543	744.5522	**-2.86**	_	_	744.5532	**-1.52**	744.5527	**-2.19**
PE(38:9)	C43H69NO8P	758.4761	758.4734	**-3.54**	758.4751	**-1.29**	758.4753	**-1.03**	758.4742	**-2.48**
PE(38:8)	C43H71NO8P	760.4917	760.4878	**-5.17**	760.4899	**-2.41**	760.4886	**-4.12**	760.4894	**-3.07**
PE(38:7)	C43H73NO8P	762.5074	762.506	**-1.81**	762.5072	**-0.24**	762.5069	**-0.63**	762.5071	**-0.37**
PE(38:6)	C43H75O8NP	764.523	764.522	**-1.35**	764.5227	**-0.43**	764.5223	**-0.96**	764.5229	**-0.17**
PE(38:5)	C43H77NO8P	766.5387	766.535	**-4.8**	766.5377	**-1.28**	766.5356	**-4.02**	766.5379	**-1.02**
PE(40:10)	C45H71NO8P	784.4917	784.4899	**-2.34**	784.4902	**-1.95**	784.49	**-2.21**	784.4901	**-2.08**
PE(40:9)	C45H73O8NP	786.5074	786.5062	**-1.5**	786.5057	**-2.14**	786.5055	**-2.39**	786.5049	**-3.16**
PE(40:8)	C45H75O8NP	788.523	788.5221	**-1.18**	788.5209	**-2.7**	788.5216	**-1.82**	788.5214	**-2.07**
PE(40:7)	C45H77NO8P	790.5387	790.5371	**-2**	790.5349	**-4.78**	790.538	**-0.86**	790.5352	**-4.4**
PE(40:1)	C45H89NO8P	802.6326	802.6302	**-2.97**	802.6298	**-3.47**	802.6345	**2.39**	802.6319	**-0.85**
LPG identified as [M−H]−										

LPG(16:0)	C22H44O9P	483.2723	483.272	**-0.62**	483.2723	**0**	483.2723	**0**	483.2725	**0.41**
				PG identified as [M−H]$^{-}$						
PG(30:1)	C36H68O10P	691.455	691.454	**-1.45**	691.4552	**0.29**	691.4549	**-0.14**	691.4555	**0.72**
PG(31:0)	C37H72O10P	707.4863	707.4855	**-1.15**	-	-	707.4863	**-0.02**	707.4865	**0.26**
PG(31:1)	C37H70O10P	705.4707	705.4694	**-1.79**	-	-	705.4702	**-0.66**	705.4702	**-0.66**
PG(32:1)	C38H72O10P	719.4863	719.4864	**0.14**	719.4874	**1.53**	719.4865	**0.28**	719.4865	**0.28**
PG(32:2)	C38H70O10P	717.4707	717.4696	**-1.53**	-	-	717.4704	**-0.42**	717.4706	**-0.14**
PG(33:1)	C39H74O10P	733.502	733.5009	**-1.45**	733.5038	**2.51**	733.5016	**-0.49**	733.5021	**0.19**
PG(34:1)	C40H76O10P	747.5176	747.5165	**-1.47**	747.5171	**-0.67**	747.5165	**-1.47**	747.5168	**-1.07**
PG(34:2)	C40H74O10P	745.502	745.5043	**3.09**	745.502	**0**	745.5022	**0.27**	745.5024	**0.54**
PG(34:5)	C40H68O10P	739.455	739.4543	**-0.95**	739.4547	**-0.41**	739.4554	**0.54**	739.4551	**0.14**
PG(35:5)	C41H70O10P	753.4707	753.4696	**-1.41**	753.4702	**-0.61**	753.4702	**-0.61**	753.4707	**0.05**
PG(36:2)	C42H78O10P	773.5333	773.5318	**-1.94**	773.5329	**-0.52**	773.5328	**-0.65**	773.5334	**0.13**
PG(36:5)	C42H72O10P	767.4863	767.4851	**-1.56**	767.4862	**-0.13**	767.4862	**-0.13**	767.4863	**0**
PG(36:5(OH))	C42H72O11P	783.4812	783.4802	**-1.31**	783.4805	**-0.93**	783.4809	**-0.42**	783.4814	**0.22**
PG(36:6)	C42H70O10P	765.4707	765.4703	**-0.52**	-	-	765.4714	**0.91**	-	-
				PI identified as [M−H]$^{-}$						
PI(28:0)	C37H70O13P	753.4554	753.4551	**-0.4**	-	-	-	-	-	-
PI(30:1)	C39H72O13P	779.4711	779.4698	**-1.67**	779.4679	**-4.11**	779.4704	**-0.9**	-	-
PI(32:1)	C41H76O13P	807.5024	807.5011	**-1.61**	807.5011	**-1.61**	807.502	**-0.5**	807.4996	**-3.47**
PI(32:2)	C41H74O13P	805.4867	805.4851	**-1.99**	805.4832	**-4.35**	805.4863	**-0.5**	805.4831	**-4.47**
PI(33:1)	C42H78O13P	821.518	821.5163	**-2.08**	821.5173	**-0.86**	821.5168	**-1.47**	821.5185	**0.6**
PI(34:1)	C43H80O13P	835.5337	835.5322	**-1.8**	835.5303	**-4.07**	835.5328	**-1.08**	835.5329	**-0.96**
PI(34:2)	C43H78O13P	833.518	833.5175	**-0.6**	833.5142	**-4.56**	833.5168	**-1.44**	833.5147	**-3.96**
PI(34:3)	C43H76O13P	831.5024	831.5014	**-1.2**	831.5015	**-1.08**	831.502	**-0.48**	-	-
PI(34:5)	C43H72O13P	827.4711	827.4703	**-0.92**	827.4711	**0.05**	827.4711	**0.05**	827.47	**-1.28**
PI(36:6)	C45H74O13P	853.4867	853.4859	**-0.94**	853.4855	**-1.41**	853.4861	**-0.7**	-	-
PI(40:10)	C49H74O13P	901.4867	901.4856	**-1.22**	901.4872	**0.55**	901.4861	**-0.67**	901.4836	**-3.44**
				PI-Cer identified as [M−H]$^{-}$						

PI-Cer(d18:1/14:0)	C38H71NO11P	750.4921	750.491	**-1.5**	750.4918	**-0.44**	-	-	750.4902	**-2.57**
PI-Cer(d18:1/14:1)	C38H73NO11P	748.4765	748.4754	**-1.47**	748.4756	**-1.2**	748.4762	**-0.4**	748.4763	**-0.27**
SQDG identified as $[M-H]^-$										
SQDG(28:0)	C37H69O12S	737.451	737.4487	**-3.09**	737.4511	**0.17**	737.4513	**0.44**	737.4513	**0.44**
SQDG(30:0)	C39H73O12S	765.4823	765.4816	**-0.88**	765.4819	**-0.49**	765.482	**-0.36**	765.4821	**-0.23**
SQDG(30:1)	C39H71O12S	763.4666	763.4659	**-0.95**	763.4662	**-0.56**	763.4664	**-0.3**	763.4663	**-0.43**
SQDG(31:1)	C40H73O12S	777.4823	777.4812	**-1.41**	777.482	**-0.39**	777.4816	**-0.9**	777.4817	**-0.77**
SQDG(32:1)	C41H75O12S	791.4979	791.4972	**-0.92**	791.4978	**-0.16**	791.4979	**-0.03**	791.4979	**-0.03**
SQDG(32:2)	C41H73O12S	789.4823	789.4812	**-1.36**	789.482	**-0.35**	789.4818	**-0.6**	789.482	**-0.35**
SQDG(32:3)	C41H71O12S	787.4666	787.4649	**-2.19**	787.4662	**-0.54**	787.4654	**-1.56**	787.4655	**-1.43**
SQDG(34:1)	C43H79O12S	819.5292	819.5282	**-1.25**	819.5288	**-0.52**	819.5293	**0.09**	819.5289	**-0.4**
SQDG(34:2)	C43H77O12S	817.5136	817.5122	**-1.68**	817.5128	**-0.95**	817.512	**-1.93**	817.5127	**-1.07**
SQDG(36:5)	C45H75O12S	839.4979	839.4959	**-2.41**	839.4969	**-1.22**	839.4972	**-0.87**	839.4971	**-0.99**
MGTS identified as $[M+H]^+$										
MGTS(14:1)	C24H46O6N	444.3325	444.3317	**-1.83**	444.3322	**-0.71**	444.3321	**-0.93**	444.3339	**3.12**
MGTS(14:0)	C24H48O6N	446.3482	446.3472	**-2.16**	446.3478	**-0.82**	446.3477	**-1.04**	446.3478	**-0.82**
MGTS(15:0)	C25H50O6N	460.3638	460.3629	**-1.99**	460.3635	**-0.68**	460.3634	**-0.9**	460.3635	**-0.68**
MGTS(16:4)	C26H44O6N	466.3169	466.3148	**-4.43**	466.3144	**-5.28**	466.3149	**-4.21**	466.3147	**-4.64**
MGTS(16:3)	C26H46O6N	468.3325	468.3302	**-4.94**	468.331	**-3.23**	468.3302	**-4.94**	468.3308	**-3.66**
MGTS(16:2)	C26H48O6N	470.3482	470.3479	**-0.56**	470.3482	**0.08**	470.3485	**0.71**	470.3483	**0.29**
MGTS(16:1)	C26H50O6N	472.3638	472.3632	**-1.3**	472.3636	**-0.45**	472.3636	**-0.45**	472.3636	**-0.45**
MGTS(16:0)	C26H52O6N	474.3795	474.3797	**0.42**	474.3795	**0**	474.3792	**-0.63**	474.3794	**-0.21**
MGTS(17:1)	C27H52O6N	486.3795	486.3787	**-1.57**	486.3792	**-0.54**	486.3787	**-1.57**	486.3792	**-0.54**
MGTS(17:2)	C27H50O6N	484.3638	484.3629	**-1.89**	484.3634	**-0.85**	484.3633	**-1.06**	484.3633	**-1.06**
MGTS(18:5)	C28H46O6N	492.3325	492.3321	**-0.84**	492.3304	**-4.29**	492.331	**-3.08**	492.3315	**-2.06**
MGTS(18:4)	C28H48O6N	494.3482	494.3474	**-1.55**	494.3457	**-4.98**	494.3457	**-4.98**	494.3461	**-4.18**
MGTS(18:3)	C28H50O6N	496.3638	496.3629	**-1.84**	496.3623	**-3.05**	496.3615	**-4.66**	496.3618	**-4.06**
MGTS(18:2)	C28H52O6N	498.3795	498.3792	**-0.53**	498.3792	**-0.53**	498.3801	**1.28**	498.3794	**-0.13**
MGTS(18:1)	C28H54O6N	500.3951	500.3952	**0.17**	500.3951	**-0.03**	500.3951	**-0.03**	500.395	**-0.23**

MGTS(20:5)	C30H50O6N	520.3638	520.3634	**-0.8**	520.3636	**-0.41**	520.3635	**-0.6**	520.3636	**-0.41**
MGTS(20:4)	C30H52O6N	522.3795	522.3791	**-0.7**	522.3792	**-0.51**	522.379	**-0.89**	522.3795	**0.07**
DGTS identified as [M+H]$^+$										
DGTS(28:1)	C38H72O7N	654.5309	654.5304	**-0.76**	654.5312	**0.46**	654.5307	**-0.31**	654.5305	**-0.61**
DGTS(28:0)	C38H74O7N	656.5465	656.5457	**-1.22**	656.5461	**-0.61**	656.5463	**-0.3**	656.5465	**0**
DGTS(30:3)	C40H72O7N	678.5309	678.5289	**-2.95**	678.5296	**-1.92**	678.5291	**-2.65**	678.5294	**-2.21**
DGTS(30:2)	C40H74O7N	680.5465	680.5456	**-1.32**	680.5461	**-0.59**	680.5461	**-0.59**	680.546	**-0.73**
DGTS(30:1)	C40H76O7N	682.5622	682.5616	**-0.88**	682.5619	**-0.44**	682.5616	**-0.88**	682.5617	**-0.73**
DGTS(32:5)	C42H72O7N	702.5309	702.529	**-2.67**	702.5286	**-3.24**	702.5298	**-1.54**	702.5291	**-2.53**
DGTS(32:4)	C42H74O7N	704.5465	704.5438	**-3.83**	704.5443	**-3.12**	704.544	**-3.55**	704.5443	**-3.12**
DGTS(32:3)	C42H76O7N	706.5622	706.5592	**-4.25**	706.5611	**-1.56**	706.5595	**-3.82**	706.5609	**-1.84**
DGTS(32:2)	C42H78O7N	708.5778	708.5768	**-1.41**	708.5775	**-0.42**	708.5774	**-0.56**	708.5774	**-0.56**
DGTS(32:1)	C42H80O7N	710.5935	710.5923	**-1.69**	710.593	**-0.7**	710.593	**-0.7**	710.5928	**-0.99**
DGTS(33:2)	C43H80O7N	722.5935	722.5926	**-1.22**	722.5932	**-0.39**	722.5926	**-1.22**	722.5925	**-1.35**
DGTS(33:1)	C43H82O7N	724.6091	724.6068	**-3.21**	724.6079	**-1.69**	724.6062	**-4.04**	724.6072	**-2.66**
DGTS(34:6)	C44H74O7N	728.5465	728.5445	**-2.75**	728.5449	**-2.2**	728.5459	**-0.82**	728.5453	**-1.65**
DGTS(34:5)	C44H76O7N	730.5622	730.5612	**-1.37**	730.5616	**-0.82**	730.5616	**-0.82**	730.5612	**-1.37**
DGTS(34:4)	C44H78O7N	732.5778	732.5751	**-3.69**	732.5759	**-2.59**	732.5743	**-4.78**	732.5757	**-2.87**
DGTS(34:3)	C44H80O7N	734.5935	734.5913	**-2.99**	734.5915	**-2.72**	734.5899	**-4.9**	734.5909	**-3.54**
DGTS(34:2)	C44H82O7N	736.6091	736.6078	**-1.76**	736.6085	**-0.81**	736.6086	**-0.68**	736.6084	**-0.95**
DGTS(34:1)	C44H84O7N	738.6248	738.6233	**-2.03**	738.6224	**-3.25**	738.6238	**-1.35**	738.6233	**-2.03**
DGTS(36:8)	C46H74O7N	752.5465	752.5427	**-5.08**	752.5433	**-4.28**	752.5434	**-4.15**	752.5435	**-4.01**
DGTS(36:7)	C46H76O7N	754.5622	754.5595	**-3.58**	754.5596	**-3.45**	754.5588	**-4.51**	754.5602	**-2.65**
DGTS(36:6)	C46H78O7N	756.5778	756.5766	**-1.59**	756.5771	**-0.93**	756.5775	**-0.4**	756.5774	**-0.53**
DGTS(36:5)	C46H80O7N	758.5935	758.592	**-1.98**	758.593	**-0.66**	758.5928	**-0.92**	758.5925	**-1.32**
DGTS(36:4)	C46H82O7N	760.6091	760.6066	**-3.29**	760.6079	**-1.58**	760.6058	**-4.34**	760.6066	**-3.29**
DGTS(36:3)	C46H84O7N	762.6248	762.6221	**-3.54**	_	_	_	_	_	_
DGTS(36:2)	C46H86O7N	764.6404	_	_	764.639	**-1.83**	764.6403	**-0.13**	764.6409	**0.65**
DGTS (37:5)	C47H82O7N	772.6091	772.6076	**-1.98**	772.6083	**-1.07**	772.6079	**-1.59**	772.6076	**-1.98**
DGTS(38:10)	C48H74O7N	776.5465	776.5435	**-3.9**	776.5438	**-3.51**	776.5446	**-2.48**	776.5449	**-2.1**

DGTS(38:9)	C48H76O7N	778.5622	778.5595	**-3.47**	778.5604	**-2.31**	778.5599	**-2.95**	778.5595	**-3.47**
DGTS(38:8)	C48H78O7N	780.5778	780.5747	**-3.97**	780.5752	**-3.33**	780.5751	**-3.46**	780.575	**-3.59**
DGTS(38:7)	C48H80O7N	782.5935	782.5909	**-3.32**	782.591	**-3.19**	782.5896	**-4.98**	782.5914	**-2.68**
DGTS(38:6)	C48H82O7N	784.6091	784.6074	**-2.17**	784.6078	**-1.66**	784.6084	**-0.89**	784.6076	**-1.91**
DGTS(38:5)	C48H84O7N	786.6248	786.6219	**-3.69**	786.623	**-2.29**	786.6216	**-4.07**	786.6215	**-4.2**
DGTS(40:10)	C50H78O7N	804.5778	804.5771	**-0.87**	804.5777	**-0.12**	804.5773	**-0.62**	804.5767	**-1.37**
DGTS(40:9)	C50H80O7N	806.5935	806.5906	**-3.6**	806.5919	**-1.98**	806.5899	**-4.46**	806.5904	**-3.84**
Cer identified as $[M+H]^+$										
Cer(d32:2)	C32H62NO3	508.473	508.4723	**-1.32**	508.4727	**-0.53**	508.4725	**-0.92**	508.4725	**-0.92**
Cer(d32:1)	C32H64NO3	510.4886	510.4883	**-0.62**	510.4881	**-1.01**	510.4896	**1.92**	510.4893	**1.34**
MGMG identified as $[M+NH_4]^+$										
MGMG(16:0)	C25H52NO9	510.3642	510.3657	**2.94**	510.3661	**3.72**	510.3649	**1.37**	510.3646	**0.78**
DGMG identified as $[M+NH_4]^+$										
DGMG(14:0)	C29H58NO14	644.3857	644.3852	**-0.83**	644.3852	**-0.83**	644.385	**-1.14**	644.3854	**-0.52**
DGMG(16:1)	C31H60NO14	670.4014	670.4009	**-0.72**	670.4014	**0.02**	670.4008	**-0.87**	670.4009	**-0.72**
DGMG(16:0)	C31H62NO14	672.417	672.4165	**-0.74**	672.4163	**-1.04**	672.4166	**-0.59**	672.4166	**-0.59**
DGMG(20:5)	C35H60NO14	718.4014	718.4009	**-0.67**	718.4012	**-0.26**	718.4013	**-0.12**	718.4005	**-1.23**
MGDG identified as $[M+NH_4]^+$										
MGDG(30:1)	C39H76NO10	718.5464	_	_	718.5469	**0.7**	718.5426	**-5.29**	718.5462	**-0.28**
MGDG(32:5)	C41H72NO10	738.5156	_	_	738.5167	**1.46**	738.5178	**2.95**	738.5163	**0.92**
MGDG(32:2)	C41H78NO10	744.5626	744.5593	**-4.43**	744.5617	**-1.21**	744.5609	**-2.28**	744.562	**-0.81**
MGDG(32:1)	C41H80NO10	746.5777	746.5765	**-1.61**	746.5778	**0.13**	746.5784	**0.94**	746.5777	**0**
MGDG(34:5)	C43H76NO10	766.5469	766.546	**-1.21**	766.546	**-1.21**	766.5468	**-0.16**	766.5467	**-0.29**
MGDG(34:2)	C43H82NO10	772.5933	_	_	772.5936	**0.39**	_	_	772.5923	**-1.29**
MGDG(34:1)	C43H84NO10	774.609	_	_	774.6085	**-0.65**	_	_	774.6083	**-0.9**
MGDG(36:6)	C45H78NO10	792.5625	792.5628	**0.38**	792.5625	**0**	792.5618	**-0.88**	792.5624	**-0.13**
MGDG(36:5)	C45H80NO10	794.5782	794.5755	**-3.4**	794.5777	**-0.63**	794.5773	**-1.13**	794.5776	**-0.76**
MGDG(38:7)	C47H80NO10	818.5782	_	_	818.5786	**0.46**	818.5775	**-0.88**	818.5781	**-0.15**
MGDG(40:10)	C49H78NO10	840.5626	840.565	**2.89**	840.5626	**0.04**	840.5618	**-0.92**	840.5625	**-0.08**

MGDG(40:9)	$C_{49}H_{80}NO_{10}$	842.5782	842.574	**-4.98**	842.576	**-2.61**	842.5739	**-5.1**	842.574	**-4.98**
MGDG(40:8)	$C_{49}H_{82}NO_{10}$	844.5939	844.593	**-1.03**	844.5927	**-1.39**	844.5917	**-2.57**	844.5934	**-0.56**
DGDG identified as $[M+NH_4]^+$										
DGDG(30:1)	$C_{45}H_{86}O_{15}N$	880.5997	880.5993	**-0.45**	880.5996	**-0.11**	880.5992	**-0.57**	880.5992	**-0.57**
DGDG(30:0)	$C_{45}H_{88}O_{15}N$	882.6154	882.6132	**-2.49**	882.6149	**-0.57**	882.6122	**-3.63**	882.6191	**4.19**
DGDG(32:5)	$C_{47}H_{82}O_{15}N$	900.5684	900.569	**0.67**	900.5696	**1.33**	900.5685	**0.11**	900.571	**2.89**
DGDG(32:3)	$C_{47}H_{86}O_{15}N$	904.5997	904.5998	**0.11**	904.6005	**0.88**	904.6028	**3.43**	904.6019	**2.43**
DGDG(32:2)	$C_{47}H_{88}O_{15}N$	906.6154	906.6153	**-0.11**	906.6153	**-0.11**	906.6158	**0.44**	906.6152	**-0.22**
DGDG(32:1)	$C_{47}H_{90}O_{15}N$	908.631	908.6299	**-1.21**	908.6306	**-0.44**	908.6302	**-0.88**	908.6302	**-0.88**
DGDG(34:5)	$C_{49}H_{86}O_{15}N$	928.5997	928.5995	**-0.22**	928.5995	**-0.22**	928.5998	**0.11**	928.6007	**1.08**
DGDG(34:3)	$C_{49}H_{90}O_{15}N$	932.631	932.631	**0**	932.6307	**-0.32**	932.6272	**-4.07**	932.6308	**-0.21**
DGDG(34:2)	$C_{49}H_{92}O_{15}N$	934.6467	934.6441	**-2.78**	934.6461	**-0.64**	934.6444	**-2.46**	934.6458	**-0.96**
DGDG(34:1)	$C_{49}H_{94}O_{15}N$	936.6623	936.6587	**-3.84**	936.6601	**-2.35**	936.6594	**-3.1**	936.6601	**-2.35**
DGDG(35:5)	$C_{50}H_{88}NO_{15}$	942.6154	942.6156	**0.21**	942.6161	**0.74**	942.6143	**-1.17**	942.6157	**0.32**
DGDG(36:7)	$C_{51}H_{86}O_{15}N$	952.5997	952.5989	**-0.84**	952.5989	**-0.84**	952.5982	**-1.57**	952.5994	**-0.31**
DGDG(36:6)	$C_{51}H_{88}O_{15}N$	954.6154	954.6152	**-0.21**	954.6152	**-0.21**	954.6155	**0.1**	954.6153	**-0.1**
DGDG(36:5)	$C_{51}H_{90}O_{15}N$	956.631	956.6283	**-2.82**	956.6305	**-0.52**	956.6301	**-0.94**	956.6303	**-0.73**
DGDG(38:7)	$C_{53}H_{90}O_{15}N$	980.631	980.6305	**-0.51**	980.6311	**0.1**	980.6305	**-0.51**	980.6296	**-1.43**
DGDG(38:6)	$C_{53}H_{92}O_{15}N$	982.6467	982.6462	**-0.51**	982.6439	**-2.85**	982.6473	**0.61**	982.6446	**-2.14**
DGDG(40:10)	$C_{55}H_{88}O_{15}N$	1002.6154	1002.615	**-0.6**	1002.616	**0.5**	1002.615	**-0.2**	1002.616	**0.4**

Abbreviations: NO, *N. oceanica*; NL, *N. limnetica*; Out, grown outdoors; In, grown indoors; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

Table S5.7. List of molecular ions identified by HILIC-ESI-MS in negative ion mode, as $[M-H]^-$ or in positive ion mode, as $[M+H]^+$ and $[M+NH_4]^+$ ions, and lipid molecular species identified by HILIC-MS/MS in the total lipid extracts of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In). The identification of the fatty acyl composition correspondent to each molecular ion was confirmed by the analysis of the LC-MS/MS spectra of each $[M+CH_3COO]^-$ ion for PC and LPC classes; $[M-H]^-$ for PG, LPG, PI, PI-Cer, SQDG, PE and LPE classes; $[M+H]^+$ for DGTS, MGTS and Cer classes; $[M+NH_4]^+$ for DGDG, DGMG, MGDG and MGMG classes, as detailed in [33,35]. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains, bold *m/z* values correspond to the most abundant molecular ions detected in each class.

		Nannochloropsis oceanica **- Outdoor**	***Nannochloropsis oceanica*** **- Indoor**	***Nannochloropsis limnetica*** **- Outdoor**	***Nannochloropsis limnetica*** **- Indoor**
Lipid species (C:N)	**Calculated *m/z***	**Fatty acyl chains observed in MS/MS of the NO-Out**	**Fatty acyl chains observed in MS/MS of the NO-In**	**Fatty acyl chains observed in MS/MS of the NL-Out**	**Fatty acyl chains observed in MS/MS of the NL-In**
		LPC identified as $[M+H]^+$			
LPC(14:0)	468.309	14:0	14:0	14:0	14:0
LPC(16:3)	490.2934	16:3	-	16:3	-
LPC(16:2)	492.309	16:2	16:2	16:2	16:2
LPC(16:1)	494.3247	**16:1**	16:1	16:1	16:1
LPC(16:0)	496.3403	16:0	16:0	16:0	16:0
LPC(17:0)	510.356	17:0	-	-	-
LPC(17:1)	508.3403	17:1	17:1	17:1	17:1
LPC(17:2)	506.3247	17:2	17:2	17:2	17:2
LPC(18:5)	514.2934	-	-	-	-
LPC(18:4)	516.309	18:4	-	18:4	-
LPC(18:3)	518.3247	18:3	18:3	18:3	18:3
LPC(18:2)	520.3403	18:2	18:2	18:2	18:2
LPC(18:1)	522.356	-	**18:1**	18:1	**18:1**
LPC(20:5)	542.3247	20:5	20:5	**20:5**	20:5

LPC(20:4)	544.3403	20:4	20:4	-	20:4
LPC(20:3)	546.356	-	-	20:3	-
LPC(20:1)	550.3873	-	20:1	-	-
LPC(20:0)	552.4029	20:0	20:0	20:0	20:0
LPC(22:6)	568.3403	-	-	-	-
LPC(22:5)	570.356	-	22:5	22:5	-
PC identified as [M+H]$^+$					
PC(28:1)	676.4917	-	-	-	-
PC(30:3)	700.4917	-	-	-	-
PC(30:1)	704.523	16:1-14:0	14:0-16:1 and 18:1-12:0	16:1-14:0 and 14:1-16:0	14:0-16:0
PC(31:2)	716.523	16:1-15:1	-	16:1-15:1, 16:2-15:0	16:1-15:1
-	-	-	-	and 17:2-14:0	-
PC(31:1)	718.5387	16:1-15:0, 17:1-14:0	16:1-15:0 and 16:0-15:1	16:1-15:0, 16:0-15:1	16:1-15:0 and 17:1-14:0
-	-	and 15:1-16:0	-	and 17:1-14:0	-
PC(32:5)	724.4917	-	-	16:1-16:4	-
PC(32:4)	726.5074	16:3-16:1 and 16:2-16:2	-	16:1-16:3, 16:0-16:4	16:3-16:1 and 16:2-16:2
-	-	-	-	and 16:2-16:2	-
PC(32:3)	728.523	16:2-16:1, 16:3-16:0	16:2-16:1	18:3-14:0, 16:2-16:1	16:2-16:1 and 16:3-16:0
-	-	and 18:3-14:0	-	and 16:3-16:0	-
PC(32:2)	730.5387	**16:1/16:1, 16:2-16:0**	16:1/16:1, 16:2-16:0,	18:2-14:0, 16:2:16:0	16:1/16:1 and 16:2-16:0
-	-	**and 18:2-14:0**	18:2-14:0 and 18:1-14:1	and 16:1-16:1	-
PC(32:1)	732.5543	-	16:1-16:0 and 18:1-14:0	16:1-16:0 and 18:1-14:0	16:1-16:0 and 18:1-14:0
PC(33:3)	742.5387	17:2-16:1, 17:1-16:2	17:2-16:1 and 17:1-16:2	17:2-16:1, 17:1-16:2	16:1-17:2 and 17:1-16:2
-	-	and 15:0-18:3	-	and 18:3-15:0	-
PC(33:2)	744.5543	18:2-15:0, 17:1-16:1	17:1-16:1, 17:2-16:0,	17:1-16:1, 17:2-16:0	17:1-16:1, 17:2-16:0
-	-	and 17:2-16:0	18:1-15:1 and 18:2-15:0	and 18:2-15:0	and 18:2-15:0
PC(34:7)	748.4917	-	-	-	-
PC(34:6)	750.5074	16:1-18:5	-	-	-
PC(34:5)	752.523	18:3-16:2, 18:4-16:1,	14:0-20:5	20:5-14:0, 18:2-16:3, 18:5-16:0,	14:0-20:5, 18:2-16:3,
-	-	14:0-20:5 and 18:2-16:3	-	18:3-16:2 and 18:4-16:1	18:3-16:2 and 16:1-18:4

PC(34:4)	754.5387	20:4-14:0, 16:1-18:3, 18:4-16:0,	18:3-16:1 and 18:2-16:2	20:4-14:0, 18:1-16:3,	18:2-16:2 and 18:3-16:1
-	-	18:2-16:2 and 18:1-16:3	-	18:2-16:2 and 16:1-18:3	-
PC(34:3)	756.5543	18:2-16:1	18:1-16:2, 18:2-16:1	20:3-14:0, 18:1-16:2,	**18:1-16:2, 18:2-16:1**
-	-	-	and 18:3-16:0	18:3-16:0 and 18:2-16:1	**and 18:3-16:0**
PC(34:2)	758.57	**18:1-16:1 and 18:2-16:0**	**18:1-16:1 and 18:2-16:0**	**18:1-16:1 and 18:2-16:0**	**18:1-16:1 and 18:2-16:0**
PC(34:1)	760.5856	18:1-16:0 and 16:1-18:0	**18:1-16:0 and 16:1-18:0**	18:1-16:0 and 16:1-18:0	18:1-16:0 and 16:1-18:0
PC(35:4)	768.5543	-	18:2-17:2 and 18:3-17:1	-	18:2-17:2 and 18:3-17:1
PC(35:3)	770.57	-	18:1-17:2 and 18:2-17:1	18:1-17:2	18:1-17:2 and 18:2-17:1
PC(35:2)	772.5856	-	18:1-17:1	18:1-17:1	18:2-17:0 and 18:1-17:1
PC(35:1)	774.6013	16:1-17:0	-	18:1-17:0	18:1-17:0 and 18:0-17:1
PC(37:7)	790.5387	17:2-20:5	-	17:2-20:5	20:5-17:2
PC(37:6)	792.5543	17:1-20:5	17:1-20:5 and 17:2-20:4	17:1-20:5, 17:2-20:4	17:1-20:5 and 17:2-20:4
-	-	and 17:2-20:4	-	and 21:5-16:1	-
PC(36:8)	774.5074	-	-	-	-
PC(36:7)	776.523	16:2-20:5	16:2-20:5	16:2-20:5 and 16:3-20:4	16:2-20:5
PC(36:6)	778.5387	16:1-20:5 and 16:2-20:4	20:5-16:1	20:5-16:1 and 20:4-16:2	16:1-20:5, 16:2-20:4,
-	-	-	-	-	18:3/18:3 and 18:2-18:4
PC(36:5)	780.5543	16:1-20:4 and 16:0-20:5	-	**20:5-16:0 and 16:1-20:4**	-
PC(36:4)	782.57	18:2/18:2, 20:3-16:1	18:2/18:2, 18:1-18:3,	-	-
-	-	and 20:4-16:0	20:4-16:0 and 20:3-16:1	-	-
PC(36:4(OH))	798.5649	-	-	-	18:2-18:2-OH
PC(36:3)	784.5856	20:3-16:0, 16:1-20:2	18:1-18:2, 20:2-16:1,	20:3-16:0, 16:1-20:2	18:1-18:2, 20:3-16:0
-	-	and 18:2-18:1	18:3-18:0 and 20:3-16:0	and 18:2-18:1	and 20:2-16:1
PC(36:2)	786.6013	18:1/18:1, 20:2-16:0,	18:1/18:1 and 18:2-18:0	18:1/18:1	18:1/18:1, 18:2-18:0
-	-	18:2-18:0 and 20:1-16:1	-	-	and 16:2-20:0
PC(37:5)	794.57	-	17:1-20:4	20:4-17:1, 20:5-17:0,	17:1-20:4
-	-	-	-	21:5-16:0 and 16:1-21:4	-
PC(37:4)	796.5856	-	-	21:4-16:0	-
PC(37:3)	798.6013	-	-	-	-

PC(37:2)	800.6169	-	-	-	-
PC(38:10)	798.5074	-	-	-	-
PC(38:9)	800.523	-	-	20:5-18:4	-
PC(38:8)	802.5387	18:3-20:5	-	18:3-20:5 and 18:4-20:4	18:3-20:5
PC(38:7)	804.5543	18:2-20:5 and 18:3-20:4	18:2-20:5 and 18:3-20:4	18:2-20:5 and 18:3-20:4	18:2-20:5 and 18:3-20:4
PC(38:6)	806.57	18:2-20:4, 18:1-20:5	18:1-20:5, 18:2-20:4	18:1-20:5, 18:2-20:4	18:2-20:4 and 18:1-20:5
-	-	and 20:3-18:3	and 20:3-18:3	and 18:3-20:3	-
PC(38:5)	808.5856	20:4-18:1	18:1-20:4, 20:5-18:0	18:1-20:4	18:1-20:4, 18:2-20:3
-	-	-	and 18:2-20:3	-	and 20:5-18:0
PC(38:3)	812.6169	-	18:1-20:2 and 20:3-18:0	18:1-20:2, 18:3-20:0	18:3-20:0, 18:2-20:1
-	-	-	-	and 20:3-18:0	and 18:1-20:2
PC(38:2)	814.6326	18:2-20:0	18:2-20:0	18:2-20:0 and 20:1-18:1	18:2-20:0
PC(38:1)	816.6482	-	18:1-20:0	18:1-20:0	18:1-20:0
PC(40:11)	824.523	-	-	-	-
PC(40:10)	826.5387	20:5/20:5	-	20:5/20:5	-
PC(40:9)	828.5543	20:5-20:4	20:5-20:4	20:4-20:5	20:4-20:5
PC(40:8)	830.57	-	-	-	-
PC(40:5)	836.6169	-	20:1-20:4	20:0-20:5	20:0-20:5
		LPE identified as [M+H]$^+$			
LPE(14:0)	426.2621	14:0	-	14:0	14:0
LPE(16:3)	448.2464	-	-	-	-
LPE(16:2)	450.2621	16:2	16:2	16:2	16:2
LPE(16:1)	452.2777	16:1	16:1	16:1	16:1
LPE(16:0)	454.2934	-	-	-	-
LPE(18:4)	474.2621	18:4	-	18:4	18:4
LPE(18:3)	476.2777	18:3	18:3	18:3	18:3
LPE(18:2)	478.2934	18:2	18:2	18:2	18:2
LPE(18:1)	480.309	-	18:1	18:1	18:1
LPE(20:5)	500.2777	20:5	20:5	20:5	20:5
LPE(20:4)	502.2934	**20:4**	**20:4**	**20:4**	**20:4**

LPE(20:3)	504.309	-	-	-	-
LPE(20:2)	506.3247	-	20:2	-	20:2
PE identified as $[M+H]^+$					
PE(30:3)	658.4448	-	-	-	-
PE(30:1)	662.4761	14:0-16:1 and 15:1-15:0	14:0-16:1 and 15:1-15:0	16:1-14:0, 16:0-14:1	14:0-16:1
-	-	-	-	and 15:1-15:0	-
PE(30:0)	664.4917	14:0-16:0 and 15:0/15:0	-	14:0-16:0	15:0/15:0 and 14:0-16:0
PE(32:4)	684.4604	-	-	-	-
PE(32:3)	686.4761	16:2-16:1	-	16:2-16:1	-
PE(32:2)	688.4917	16:1/16:1 and 16:2-16:0	16:1/16:1 and 16:0-16:2	**14:0-18:2 and 16:1-16:1**	16:1/16:1 and 16:2-16:0
PE(32:1)	690.5074	-	17:1-15:0, 14:0-18:1	16:0-16:1	16:0-16:1, 14:0-18:1
-	-		and 16:0-16:1		and 17:1-15:0
PE(34:6)	708.4604	-	-	-	-
PE(34:5)	710.4761	-	-	-	-
PE(34:4)	712.4917	18:3-16:1 and 14:0-20:4	-	14:0-20:4 and 18:3-16:1	-
PE(34:3)	714.5074	18:2-16:1 and 20:3-14:0	18:2-16:1	18:2-16:1, 18:3-16:0,	18:2-16:1
-	-	-	-	18:1-16:2 and 20:3-14:0	-
PE(34:2)	716.523	16:1-18:1	18:1-16:1 and 18:2-16:0	16:1-18:1, 17:1/17:1	17:1/17:1, 16:1-18:1
-	-	-	-	and 18:2-16:0	and 18:2-16:0
PE(36:8)	732.4604	-	-	-	-
PE(36:7)	734.4761	-	-	16:2-20:5	-
PE(36:6)	736.4917	16:1-20:5 and 16:2-20:4	16:1-20:5 and 16:2-20:4	16:1-20:5 and 16:2-20:4	16:1-20:5 and 16:2-20:4
PE(36:5)	738.5074	16:1-20:4	16:1-20:4 and 16:0-20:5	**16:1-20:4 and 16:0-20:5**	-
PE(36:4)	740.523	20:3-16:1 and 20:4-16:0	-	20:3-16:1 and 16:0-20:4	16:0-20:4
PE(36:3)	742.5387	-	18:1-18:2 and 16:0-20:3	18:2-18:1, 20:3-16:0	18:2-18:1, 16:1-20:2
-	-	-	-	and 16:1-20:2	and 16:0-20:3
PE(36:2)	744.5543	18:1/18:1	-	18:1/18:1	18:1/18:1
PE(38:9)	758.4761	-	-	-	-
PE(38:8)	760.4917	18:4-20:4	-	18:3-20:5 and 20:4-18:4	18:3-20:5 and 18:4-20:4
PE(38:7)	762.5074	18:3-20:4, 20:3-18:4	18:2-20:5	18:2-20:5 and 18:3-20:4	-

-	-	and 18:2-20:5	-	-	-
PE(38:6)	764.523	18:2-20:4	**18:1-20:5 and 18:2-20:4**	18:2-20:4 and 18:1-20:5	18:2-20:4 and 18:1-20:5
PE(38:5)	766.5387	18:1-20:4 and 18:0-20:5	**18:1-20:4**	18:1-20:4	18:1-20:4
PE(40:10)	784.4917	20:5/20:5	20:5/20:5	20:5/20:5	20:5/20:5
PE(40:9)	786.5074	20:4-20:5	20:4-20:5	-	**20:4-20:5**
PE(40:8)	788.523	**20:4/20:4**	20:4/20:4 and 20:3-20:5	20:4/20:4 and 20:3-20:5	**20:4/20:4 and 20:3-20:5**
PE(40:7)	790.5387	**20:3-20:4**	20:5-20:2 and 20:3-20:4	20:3-20:4 and 20:5-20:2	20:3-20:4
PE(40:1)	802.6326	-	-	-	-
LPG identified as [M−H]$^-$					
LPG(16:0)	483.2723	-	-	-	-
PG identified as [M−H]$^-$					
PG(30:1)	691.455	14:0-16:1	-	14:0-16:1	14:0-16:1
PG(31:0)	707.4863	15:0-16:0	-	15:0-16:0	-
PG(31:1)	705.4707	15:0-16:1 and 15:1-16:0	-	-	-
PG(32:1)	719.4863	16:0-16:1 and 14:0-18:1	**14:0-18:1 and 16:0-16:1**	16:0-16:1 and 14:0-18:1	**16:0-16:1 and 14:0-18:1**
PG(32:2)	717.4707	16:1/16:1 and 16:0-16:2	-	16:1/16:1 and 16:2-16:0	-
PG(33:1)	733.502	16:1-17:0	-	-	-
PG(34:1)	747.5176	16:0-18:1	16:0-18:1	-	16:0-18:1
PG(34:2)	745.502	16:0-18:2 and 16:1-18:1	16:0-18:2 and 18:1-16:1	16:1-18:1 and 16:0-18:2	16:0-18:2 and 16:1-18:1
PG(34:5)	739.455	14:0-20:5	-	14:0-20:5	14:0-20:5
PG(35:5)	753.4707	15:0-20:5	15:0-20:5	15:0-20:5	-
PG(36:2)	773.5333	-	-	18:1-18:1	-
PG(36:5)	767.4863	**16:0-20:5 and 16:1-20:4**	**-**	**16:0-20:5**	**16:0-20:5**
PG(36:5(OH))	783.4812	(16:0-OH)-20:5	(16:0-OH)-20:5	(16:0-OH)-20:5	(16:0-OH)-20:5
PG(36:6)	765.4707	**20:5-16:1**	-	**20:5-16:1**	-
PI identified as [M−H]$^-$					
PI(28:0)	753.4554	14:0/14:0	-	-	**-**

PI(30:1)	779.4711	**16:1-14:0**	16:1-14:0	16:1-14:0	-
PI(32:1)	807.5024	**16:1-16:0 and 18:1-14:0**	**14:0-18:1 and 16:1-16:0**	**14:0-18:1 and 16:1-16:0**	**16:1-16:0**
PI(32:2)	805.4867	16:1/16:1, 16:0-16:2	14:0-18:2, 16:1/16:1	14:0-18:2, 16:1/16:1	**16:1/16:1**
-	-	and 18:2-14:0	and 16:0-16:2	and 16:0-16:2	-
PI(33:1)	821.518	16:1-17:0 and 16:0-17:1	-	-	15:0-18:1, 16:0-17:1
-	-	-	-	-	and 16:1-17:0
PI(34:1)	835.5337	18:1-16:0 and 16:1-18:0	**16:0-18:1**	**16:0-18:1**	16:0-18:1
PI(34:2)	833.518	18:2-16:0 and 16:1-18:1	16:1-18:1 and 18:2-16:0	16:1-18:1 and 18:2-16:0	16:1-18:1 and 18:2-16:0
PI(34:3)	831.5024	18:2-16:1 and 16:2-18:1	-	-	-
PI(34:5)	827.4711	14:0-20:5	14:0-20:5	14:0-20:5	-
PI(36:6)	853.4867	20:5-16:1	-	-	-
PI(40:10)	901.4867	20:5/20:5	-	-	-
PI-Cer identified as $[M-H]^-$					
PI-Cer(d18:1/14:0)	750.4921	d18:1/14:0	d18:1/14:0	-	-
PI-Cer(d18:1/14:1)	748.4765	d18:1/14:1	d18:1/14:1	d18:1/14:1	d18:1/14:1
SQDG identified as $[M-H]^-$					
SQDG(28:0)	737.451	14:0/14:0	-	-	14:0/14:0
SQDG(30:0)	765.4823	**16:0-14:0**	**16:0-14:0**	**16:0-14:0**	**16:0-14:0**
SQDG(30:1)	763.4666	16:1-14:0	14:0-16:1	14:0-16:1	14:0-16:1
SQDG(31:1)	777.4823	15:0-16:1	16:1-15:0	16:1-15:0	16:1-15:0
SQDG(32:1)	791.4979	**-**	**16:1-16:0**	**16:1-16:0**	**-**
SQDG(32:2)	789.4823	16:1/16:1	16:1/16:1	16:1/16:1	16:1/16:1
SQDG(32:3)	787.4666	-	-	-	16:1-16:2
SQDG(34:1)	819.5292	16:0-18:1	16:0-18:1	16:0-18:1	18:1-16:0
SQDG(34:2)	817.5136	16:0-18:2	16:0-18:2	16:0-18:2	-
SQDG(36:5)	839.4979	-	-	-	-
MGTS identified as $[M+H]^+$					
MGTS(14:1)	444.3325	14:1	14:1	14:1	-

MGTS(14:0)	446.3482	14:0	14:0	14:0	14:0
MGTS(15:0)	460.3638	15:0	15:0	15:0	15:0
MGTS(16:4)	466.3169	16:4	16:4	16:4	-
MGTS(16:3)	468.3325	16:3	16:3	16:3	16:3
MGTS(16:2)	470.3482	-	-	16:2	-
MGTS(16:1)	472.3638	16:1	16:1	16:1	16:1
MGTS(16:0)	474.3795	-	-	16:0	16:0
MGTS(17:1)	486.3795	17:1	-	17:1	-
MGTS(17:2)	484.3638	17:2	17:2	-	17:2
MGTS(18:5)	492.3325	18:5	18:5	18:5	18:5
MGTS(18:4)	494.3482	18:4	18:4	18:4	18:4
MGTS(18:3)	496.3638	18:3	18:3	18:3	18:3
MGTS(18:2)	498.3795	18:2	18:2	-	18:2
MGTS(18:1)	500.3951	18:1	18:1	18:1	18:1
MGTS(20:5)	520.3638	**20:5**	**20:5**	**20:5**	**20:5**
MGTS(20:4)	522.3795	-	20:4	20:4	-
DGTS identified as $[M+H]^+$					
DGTS(28:1)	654.5309	16:1-12:0 and 14:1-14:0	-	-	-
DGTS(28:0)	656.5465	14:0/14:0 and 12:0-16:0	14:0/14:0 and 12:0-16:0	14:0/14:0 and 12:0-16:0	12:0-16:0 and 14:0/14:0
DGTS(30:3)	678.5309	16:3-.14:0	-	16:3-.14:0	-
DGTS(30:2)	680.5465	16:2-14:0 and 16:1-14:1	16:2-14:0 and 16:1-14:1	16:2-14:0	16:2-14:0
DGTS(30:1)	682.5622	**16:1-14:0**	16:1-14:0	16:1-14:0	16:1-14:0
DGTS(32:5)	702.5309	20:5-12:0	20:5-12:0	-	-
DGTS(32:4)	704.5465	18:4-14:0	18:4-14:0	18:4-14:0	18:4-14:0 and 20:4-12:0
DGTS(32:3)	706.5622	-	-	-	18:3-14:0
DGTS(32:2)	708.5778	16:1/16:1; 18:2-14:0	18:2-14:0; 16:1/16:1	16:1/16:1; 18:2-14:0	16:1/16:1; 18:2-14:0
-	-	and 16:2-16:0	and 16:2-16:0	and 16:2-16:0	and 16:2-16:0
DGTS(32:1)	710.5935	16:1-16:0 and 18:1-14:0	**16:1-16:0 and 18:1-14:0**	16:1-16:0 and 18:1-14:0	16:1-16:0 and 18:1-14:0
DGTS(33:2)	722.5935	17:1-16:1; 18:2-15:0	17:2-16:0; 18:2-15:0	-	17:1-16:1 and 17:2-16:0

-	-	and 17:2-16:0	and 17:1-16:1	-	-
DGTS(33:1)	724.6091	-	-	-	-
DGTS(34:6)	728.5465	20:5-14:1	-	20:5-14:1	20:5-14:1
DGTS(34:5)	730.5622	20:5-14:0	20:5-14:0	20:5-14:0	20:5-14:0
DGTS(34:4)	732.5778	20:4-14:0	20:4-14:0	20:4-14:0	20:4-14:0
DGTS(34:3)	734.5935	20:3-14:0; 16:1-18:2	18:3-16:0; 16:1-18:2	18:3-16:0 and 16:1-18:2	18:3-16:0; 16:1-18:2
-	-	and 18:3-16:0	and 20:3-14:0	-	and 20:3-14:0
DGTS(34:2)	736.6091	18:2-16:0; 18:1-16:1	18:2-16:0 and 16:1-18:1	18:2-16:0 and 16:1-18:1	18:2-16:0 and 16:1-18:1
-	-	and 20:2-14:0	-	-	-
DGTS(34:1)	738.6248	20:1-14:0 and 16:0-18:1	20:1-14:0 and 18:1-16:0	16:0-18:1	-
DGTS(36:8)	752.5465	-	-	-	-
DGTS(36:7)	754.5622	16:2-20:5	16:2-20:5	-	-
DGTS(36:6)	756.5778	16:1-20:5	16:1-20:5	20:5-16:1	20:5-16:1
DGTS(36:5)	758.5935	20:5-16:0	**20:5-16:0**	**20:5-16:0**	**20:5-16:0**
DGTS(36:4)	760.6091	-	20:4-16:0 and 18:2-18:2	_	_
DGTS(36:3)	762.6248	20:3-16:0; 20:2-16:1	_	_	_
-	-	and 18:2-18:1	-	-	-
DGTS(36:2)	764.6404	_	18:1/18:1	_	_
DGTS (37:5)	772.6091	20:5-17:0 and 20:4-17:1	-	20:5-17:0 and 20:4-17:1	-
DGTS(38:10)	776.5465	18:5-20:5	-	18:5-20:5	18:5-20:5
DGTS(38:9)	778.5622	18:4-20:5	18:4-20:5	18:4-20:5	_
DGTS(38:8)	780.5778	-	18:3-20:5	20:5-18:3	18:3-20:5
DGTS(38:7)	782.5935	20:5-18:2 and 18:3-20:4	18:2-20:5 and 18:3-20:4	-	20:5-18:2 and 18:3-20:4
DGTS(38:6)	784.6091	-	-	20:5-18:1 and 20:4-18:2	20:5-18:1 and 20:4-18:2
DGTS(38:5)	786.6248	20:4-18:1 and 20:5-18:0	-	20:4-18:1 and 20:5-18:0	20:4-18:1 and 20:5-18:0
DGTS(40:10)	804.5778	**20:5/20:5**	20:5/20:5	**20:5/20:5**	**20:5/20:5**
DGTS(40:9)	806.5935	-	20:4-20:5	-	20:5-20:4
Cer identified as $[M+H]^+$					
Cer(d32:2)	508.473	**d18:1/14:1**	-	**d18:1/14:1**	**d18:1/14:1**
Cer(d32:1)	510.4886	-	**d18:1/14:0**	-	-

MGMG identified as [M+NH$_4$]$^+$					
MGMG(16:0)	510.3642	-	16:00		
DGMG identified as [M+NH$_4$]$^+$					
DGMG(14:0)	644.3857	-	-	-	-
DGMG(16:1)	670.4014	-	-	-	-
DGMG(16:0)	672.417	**16:0**	**-**	**16:0**	**16:0**
DGMG(20:5)	718.4014	-	-	-	-
MGDG identified as [M+NH$_4$]$^+$					
MGDG(30:1)	718.5464	-	-	-	14:0-16:1
MGDG(32:5)	738.5156	-	12:0-20:5	12:0-20:5	12:0-20:5
MGDG(32:2)	744.5626	-	16:1/16:1 and 16:0-16:2	-	16:1/16:1 and 16:0-16:2
MGDG(32:1)	746.5777	-	**16:0-16:1 and 14:0-18:1**	-	16:0-16:1 and 14:0-18:1
MGDG(34:5)	766.5469	14:0-20:5 and 16:1-18:4	14:0-20:5 and 16:1-18:4	14:0-20:5	**14:0-20:5 and 16:1-18:4**
MGDG(34:2)	772.5933	-	16:0-18:2 and 16:1-18:1	-	-
MGDG(34:1)	774.609	-	16:0-18:1	-	16:0-18:1
MGDG(36:6)	792.5625	16:1-20:5	16:1-20:5	16:1-20:5	16:1-20:5
MGDG(36:5)	794.5782	-	16:0-20:5 and 16:1-20:4	16:0-20:5 and 16:1-20:4	16:0-20:5 and 16:1-20:4
MGDG(38:7)	818.5782	-	18:2-20:5 and 16:1-22:6	18:2-20:5 and 16:1-22:6	18:2-20:5 and 16:1-22:6
MGDG(40:10)	840.5626	**-**	20:5/20:5	**20:5/20:5**	20:5/20:5
MGDG(40:9)	842.5782	-	20:4-20:5	20:4-20:5	20:4-20:5
MGDG(40:8)	844.5939	-	20:4/20:4	20:4/20:4	20:4/20:4
DGDG identified as [M+NH$_4$]$^+$					
DGDG(30:1)	880.5997	14:0-16:1	14:0-16:1	-	14:0-16:1
DGDG(30:0)	882.6154	-	14:0-16:0	-	14:0-16:0
DGDG(32:5)	900.5684	-	-	-	-
DGDG(32:3)	904.5997	-	16:1-16:2	16:1-16:2	16:1-16:2
DGDG(32:2)	906.6154	16:1/16:1; 14:0-18:2	16:1/16:1; 16:0-16:2	16:1/16:1; 16:0-16:2	16:1/16:1; 16:0-16:2
-	-	and 16:0-16:2	and 14:0-18:2	and 14:0-18:2	and 14:0-18:2

DGDG(32:1)	908.631	16:0-16:1	**16:0-16:1 and 14:0-18:1**	16:0-16:1 and 14:0-18:1	**16:0-16:1 and 14:0-18:1**
DGDG(34:5)	928.5997	14:0-20:5	14:0-20:5	14:0-20:5	14:0-20:5
DGDG(34:3)	932.631	-	16:1-18:2 and 16:0-18:3	-	-
DGDG(34:2)	934.6467	-	16:0-18:2; 16:1-18:1	-	16:0-18:2; 16:1-18:1
-	-	-	and 14:0-20:2		and 14:0-20:2
DGDG(34:1)	936.6623	-	-	-	16:0-18:1 and 16:1-18:0
DGDG(35:5)	942.6154	15:0-20:5	-	15:0-20:5	-
DGDG(36:7)	952.5997	-	16:2-20:5	-	16:2-20:5
DGDG(36:6)	954.6154	**16:1-20:5**	16:1-20:5	**16:1-20:5**	16:1-20:5
DGDG(36:5)	956.631	**-**	**16:0-20:5 and 16:1-20:4**	**16:0-20:5 and 16:1-20:4**	**16:0-20:5 and 16:1-20:4**
DGDG(38:7)	980.631	18:2-20:5	18:2-20:5	-	-
DGDG(38:6)	982.6467	-	-	-	-
DGDG(40:10)	1002.615	20:5/20:5	20:5/20:5	20:5/20:5	20:5/20:5

Abbreviations: NO, *N. oceanica*; NL, *N. limnetica*; Out, grown outdoors; In, grown indoors; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

Table S5.8. Assignment of polar lipids identified in the total lipid extracts of *N. oceanica* (NO) grown outdoors (Out) was confirmed by the presence of fragment ions and neutral losses of the polar heads and acyl side chains, as detailed in [33,35]. Briefly, the fatty acyl composition of PC, LPC, PG, LPG, PE, LPE, PI, and SQDG classes were assigned by the $RCOO^-$ fragment ions (in negative ion mode), DGTS and MGTS classes by the deduction by the losses of fatty acyl chains as acid (RCOOH), represented without italic and keto (R=C=O), represented in italic (in positive ion mode), DGDG, MGDG, DGMG and MGMG classes by the presence of product ions corresponding to each fatty acyl group as an acylium ion plus 74 (RCO + 74) (in positive ion mode).

***Nannochloropsis oceanica* - Outdoor**				
Lipid species (C:N)	**Calculated *m/z***	**Fatty acyl chains (C:N)**	**Polar head *m/z* and neutral loss**	**Fatty acyl chains *m/z***
		in MS/MS of the NO-Out	**in MS/MS of the NO-Out**	**in MS/MS of the NO-Out**
LPC identified as $[M+H]^+$				
LPC(14:0)	468.309	14:0	184.0729	227.2007
LPC(16:3)	490.2934	16:3	184.0731	249.1856
LPC(16:2)	492.309	16:2	184.073	251.2013
LPC(16:1)	494.3247	16:1	184.0731	253.217
LPC(16:0)	496.3403	16:0	184.073	255.2326
LPC(17:0)	510.356	17:0	184.073	269.2489
LPC(17:1)	508.3403	17:1	184.0729	267.2328
LPC(17:2)	506.3247	17:2	184.0729	265.2168
LPC(18:5)	514.2934	-	-	-
LPC(18:4)	516.309	18:4	184.0729	275.2019
LPC(18:3)	518.3247	18:3	184.073	277.2169
LPC(18:2)	520.3403	18:2	184.073	279.2326
LPC(18:1)	522.356	18:1	-	281.2483
LPC(20:5)	542.3247	20:5	184.0731	301.2169
LPC(20:4)	544.3403	20:4	184.0731	303.2327
LPC(20:3)	546.356	-	-	-
LPC(20:1)	550.3873	-	-	-
LPC(20:0)	552.4029	20:0	184.073	311.2955
LPC(22:6)	568.3403	-	-	-
LPC(22:5)	570.356	-	-	-
PC identified as $[M+H]^+$				
PC(28:1)	676.4917	-	184.073	-
PC(30:3)	700.4917	-	184.0732	-
PC(30:1)	704.523	16:1-14:0	184.073	253.170-227.2010
		-		-
PC(31:2)	716.523	16:1-15:1	184.073	253.2167-239.2012
		-		-
		-		-
PC(31:1)	718.5387	16:1-15:0	184.073	253.2171-241.2169
		17:1-14:0		267.2333-227.2007

		15:1-16:0		239.2012-255.2336
PC(32:5)	724.4917	-	184.073	-
PC(32:4)	726.5074	16:3-16:1	184.073	249.1858-253.2168
		16:2/16:2		251.2013-521.2013
		-		-
PC(32:3)	728.523	16:2-16:1	184.0731	251.2012-253.2168
		16:3-16:0		249.1852-255.2322
		18:3-14:0		277.2169-227.2001
PC(32:2)	730.5387	16:1/16:1	184.073	253.2169-253.2169
		16:2-16:0		251.2014-255.2330
		18:2-14:0		279.2324-227.2016
		-		-
PC(32:1)	732.5543	-	184.073	-
		-		-
PC(33:3)	742.5387	17:2-16:1	184.0731	265.2171-253.2170
		17:1-16:2		267.2335- 251.2019
		15:0-18:3		241.2156-277.2152
PC(33:2)	744.5543	18:2-15:0	184.0731	279.2326-241.2173
		17:1-16:1		267.2327-253.2169
		17:2-16:0		265.2169-255.2333
		-		-
PC(34:7)	748.4917	-	184.073	-
PC(34:6)	750.5074	16:1-18:5	184.073	253.2174-273.1854
PC(34:5)	752.523	18:3-16:2	184.0729	277.2177-251.2017
		18:4-16:1		275.2017-253.2171
		14:0-20:5		227.2011-301.2168
		18:2-16:3		279.2323-249.1847
		-		-
PC(34:4)	754.5387	20:4-14:0	184.073	303.2330-227.2012
		16:1-18:3		253.2169-277.2170
		18:4-16:0		275.2010-255.2328
		18:2-16:2		279.2321-251.2002
		18:1-16:3		281.2480-249.1857
PC(34:3)	756.5543	18:2-16:1	184.073	279.2322-253.2163
		-		-
		-		-
		-		-
PC(34:2)	758.57	18:1-16:1	184.073	281.2483-253.2168
		18:2-16:0		279.2330-255.2324
PC(34:1)	760.5856	18:1-16:0	184.073	281.2484-255.2327
		16:1-18:0		253.2170-283.2546
PC(35:4)	768.5543	-	184.073	-
		-		-
PC(35:3)	770.57	-	184.073	-
		-		-
PC(35:2)	772.5856	-	184.073	-

		-		-
PC(35:1)	774.6013	16:1-17:0	184.073	253.2181-269.2119
		-		-
PC(37:7)	790.5387	17:2-20:5	184.073	265.2176-301.2173
PC(37:6)	792.5543	21:5-16:1	184.0727	315.3239-253.2169
		17:1-20:5		267.2325-301.2158
		17:2-20:4		265.2172-303.2310
PC(36:8)	774.5074	-	184.073	-
PC(36:7)	776.523	16:2-20:5	184.073	251.2010-301.2169
		-		-
PC(36:6)	778.5387	16:1-20:5	184.073	253.2168-301.2169
		16:2-20:4		251.2012-303.2332
		-		-
		-		-
PC(36:5)	780.5543	16:1-20:4	184.0731	253.2172-303.2338
		16:0-20:5		255.2333-301.2174
		-		-
PC(36:4)	782.57	18:2-18:2	184.0731	279.2326-279.2326
		20:3-16:1		305.2484-253.2169
		20:4-16:0		303.2328-255.2329
		-		-
PC(36:4(OH))	798.5649	-	184.073	-
PC(36:3)	784.5856	20:3-16:0	184.073	305.2484-255.2327
		16:1-20:2		253.2166-307.2556
		18:2-18:1		279.2327-281.2483
		-		-
PC(36:2)	786.6013	18:1-18:1	184.073	281.2484-281.2484
		20:2-16:0		307.2628-255.2329
		18:2-18:0		279.2326-283.2554
		20:1-16:1		309.2797-253.2165
PC(37:5)	794.57	-	184.0731	-
		-		-
		-		-
		-		-
PC(37:4)	796.5856	-	184.0732	-
PC(37:3)	798.6013	-	184.073	-
PC(37:2)	800.6169	-	184.073	-
PC(38:10)	798.5074	-	184.073	-
PC(38:9)	800.523	-	184.073	-
PC(38:8)	802.5387	18:3-20:5	184.073	277.2173-301.2174
		-		
PC(38:7)	804.5543	18:2-20:5	184.073	279.2325-301.2169
		18:3-20:4		277.2169-303.2323
PC(38:6)	806.57	18:2-20:4	184.073	279.2326-303.232
		18:1-20:5		281.2482-301.2169
		20:3-18:3		305.2464-277.2167

PC(38:5)	808.5856	20:4-18:1	184.073	303.2326-281.2491
		-		-
		-		-
PC(38:3)	812.6169	-	184.073	-
		-		-
		-		-
PC(38:2)	814.6326	18:2-20:0	184.073	279.2333-311.2969
		-		-
PC(38:1)	816.6482	-	184.073	-
PC(40:11)	824.523	-	184.0729	-
PC(40:10)	826.5387	20:5/20:5	184.073	301.2171-301.2171
PC(40:9)	828.5543	20:5-20:4	184.073	301.2169-303.2326
PC(40:8)	830.57	-	184.073	-
PC(40:5)	836.6169	-	184.0731	-
LPE identified as [M+H]$^+$				
LPE(14:0)	426.2621	14:0	285.2413	227.201
LPE(16:3)	448.2464	-	-	-
LPE(16:2)	450.2621	16:2	309.2408	251.2012
LPE(16:1)	452.2777	16:1	311.2571	253.2169
LPE(16:0)	454.2934	-	313.2717	-
LPE(18:4)	474.2621	18:4	333.2397	275.2016
LPE(18:3)	476.2777	18:3	335.2571	277.2168
LPE(18:2)	478.2934	18:2	337.2727	279.2326
LPE(18:1)	480.309	18:1	-	281.2486
LPE(20:5)	500.2777	20:5	359.2568	301.2175
LPE(20:4)	502.2934	20:4	361.272	303.2326
LPE(20:3)	504.309	-	363.2879	-
LPE(20:2)	506.3247	-	-	-
PE identified as [M+H]$^+$				
PE(30:3)	658.4448	-	517.4213	-
PE(30:1)	662.4761	14:0-16:1	521.4542	227.2010-253.2168
		15:1-15:0		239.2011-241.2165
		-		-
PE(30:0)	664.4917	14:0-16:0	-	227.2008-255.2332
		15:0/15:0		241.2168-241.2168
PE(32:4)	684.4604	-	543.437	
PE(32:3)	686.4761	16:2-16:1	-	251.2014-253.2169
PE(32:2)	688.4917	16:1/16:1	547.4707	253.2171-253.2171
		16:2-16:0		251.2012-255.2326
PE(32:1)	690.5074	-	549.4861	-
		-		-
		-		-
PE(34:6)	708.4604	-	-	-
PE(34:5)	710.4761	-	-	-
PE(34:4)	712.4917	18:3-16:1	571.4685	277.2171-253.2160

		14:0-20:4		227.2008-303.2331
PE(34:3)	714.5074	18:2-16:1	573.4869	279.2329-253.2167
		20:3-14:0		305.2494-227.2015
		-		-
		-		-
PE(34:2)	716.523	16:1-18:1	575.502	253.2170-281.2484
		-		-
		-		-
PE(36:8)	732.4604	-	-	-
PE(36:7)	734.4761	-	-	-
PE(36:6)	736.4917	16:1-20:5	595.4692	253.2168-301.2169
		16:2-20:4		251.2012-303.2320
PE(36:5)	738.5074	16:1-20:4	597.4849	253.2168-303.2327
		-		-
		-		-
PE(36:4)	740.523	20:3-16:1	599.5013	305.2483-253.2168
		20:4-16:0		303.2324-255.2309
PE(36:3)	742.5387	-	-	-
		-		-
		-		-
PE(36:2)	744.5543	18:1/18:1	-	281.2477-281.2477
PE(38:9)	758.4761	-	617.452	-
PE(38:8)	760.4917	18:4-20:4	619.4684	275.2018-303.2325
		-		-
PE(38:7)	762.5074	18:3-20:4	621.4844	277.2169-303.2326
		20:3-18:4		305.2481-275.2010
		18:2-20:5		279.2326-301.2169
PE(38:6)	764.523	18:2-20:4	623.5003	279.2328-303.2332
		-		-
PE(38:5)	766.5387	18:1-20:4	625.5226	281.2480-303.2325
PE(40:10)	784.4917	20:5/20:5	643.4675	301.2169-301.2169
PE(40:9)	786.5074	20:4-20:5	645.485	303.2325-301.2167
PE(40:8)	788.523	20:4-20:4	647.5006	303.2324-303.2324
		-		-
PE(40:7)	790.5387	20:3-20:4	649.5164	305.2484-303.2326
		20:5-20:2		301.2172-307.2626
PE(40:1)	802.6326	-	-	-
LPG identified as $[M-H]^-$				
LPG(16:0)	483.2723	16:0	-	-
PG identified as $[M-H]^-$				
PG(30:1)	691.455	14:0-16:1	152.9946, 171.0052	227.2010-253.2166
PG(31:0)	707.4863	15:0-16:0	152.9945, 171.0057	241.2168-255.2324
PG(31:1)	705.4707	15:0-16:1	152.995	241.2167-253.2157
		15:1-16:0		239.2019-255.2333
PG(32:1)	719.4863	16:0-16:1	152.9946, 171.0051, 227.0315	255.2324-253.2167

		14:0-18:1		227.2008-281.2490
PG(32:2)	717.4707	16:1/16:1	152.9949, 171.0051, 227.0323	253.2169-253.2169
		16:0-16:2		255.2322-251.2012
PG(33:1)	733.502	16:1-17:0	152.9947	253.2168-269.2473
PG(34:1)	747.5176	16:0-18:1	152.9939	255.2319-281.2507
PG(34:2)	745.502	16:0-18:2	152.9944, 171.0053	255.2325-279.2328
		16:1-18:1		253.2168-281.2483
PG(34:5)	739.455	14:0-20:5	152.9947, 171.0050, 227.0321	227.2009-301.2166
PG(35:5)	753.4707	15:0-20:5	152.9946, 171.0042	241.2167-301.2164
PG(36:2)	773.5333	-		-
PG(36:5)	767.4863	16:0-20:5	152.9946, 171.0053, 227.0320	255,2325-301.2169
		16:1-20:4		253.2169-303.2248
PG(36:5(OH))	783.4812	(16:0-OH)-20:5	152.9946, 171.0052, 227.0318	271.2278-301.2169
PG(36:6)	765.4707	20:5-16:1	152.9946, 171.0052, 227.0319	301.2171-253.2169
		-		-
PI identified as [M−H]$^-$				
PI(28:0)	753.4554	14:0/14:0	152.9946, 241.0115	227.2013-227.2013
PI(30:1)	779.4711	16:1-14:0	152.9946, 241.0114	253.2169-227.2010
PI(32:1)	807.5024	16:1-16:0	152.9947, 241.0114	253.2169-255.2326
	807.5024	18:1-14:0		281.2482-227.2007
PI(32:2)	805.4867	16:1/16:1	152.9950, 241.0114	253.2169-253.2169
	805.4867	16:0-16:2		255.2328-251.2005
	805.4867	18:2-14:0		279.2327-227.2010
PI(33:1)	821.518	16:1-17:0	152.9945-241.0114	253.2169-269.2486
	821.518	16:0-17:1		255.2325-267.1967
	821.518	-		-
PI(34:1)	835.5337	18:1-16:0	152.9946, 241.0115	281.2483-255.2326
	835.5337	16:1-18:0		253.2165-283.2618
PI(34:2)	833.518	18:2-16:0	152.9946, 241.0113	279.2325-255.2326
	833.518	16:1-18:1		253.2168-281.2481
PI(34:3)	831.5024	18:2-16:1	152.9946, 241.0114	279.2326-253.2169
	831.5024	16:2-18:1		251.2014-281.2488
PI(34:5)	827.4711	14:0-20:5	152.9947, 241.0114	227.2008-301.2169
PI(36:6)	853.4867	20:5-16:1	152.9945, 241.0113	301.2169-253.2169
PI(40:10)	901.4867	20:5/20:5	152.9948, 241.0116	301.2172-301.2172
PI-Cer identified as [M−H]$^-$				
PI-Cer(d18:1/14:0)	750.4921	d18:1/14:0	241.0113, 259.0228	-
PI-Cer(d18:1/14:1)	748.4765	d18:1/14:1	241.0113, 259.0220	-
SQDG identified as [M−H]$^-$				
SQDG(28:0)	737.451	14:0/14:0	225.0069	227.2018
SQDG(30:0)	765.4823	16:0-14:0	225.0068	255.2328-227.2019
SQDG(30:1)	763.4666	16:1-14:0	225.0068	253.2166-227.2010

SQDG(31:1)	777.4823	15:0-16:1	225.0068	241.2165-253.2171
SQDG(32:1)	791.4979	-	225.0068	-
SQDG(32:2)	789.4823	16:1/16:1	225.0068	253.2171
SQDG(32:3)	787.4666	-	225.0076	-
SQDG(34:1)	819.5292	16:0-18:1	225.007	255.2330-281.2486
SQDG(34:2)	817.5136	16:0-18:2	225.0067	255.2327-279.2324
SQDG(36:5)	839.4979	-	225.0069	-
MGTS identified as [M+H]$^+$				
MGTS(14:1)	444.3325	14:1	236.1485	236.1485
MGTS(14:0)	446.3482	14:0	236.1486	236.1486
MGTS(15:0)	460.3638	15:0	236.1488	236.1488
MGTS(16:4)	466.3169	16:4	236.1482	236.1482
MGTS(16:3)	468.3325	16:3	236.1486	236.1486
MGTS(16:2)	470.3482	-	-	-
MGTS(16:1)	472.3638	16:1	236.1486	236.1486
MGTS(16:0)	474.3795	-	-	-
MGTS (17:1)	486.3795	17:1	236.1486	236.1486
MGTS (17:2)	484.3638	17:2	236.1486	236.1486
MGTS(18:5)	492.3325	18:5	236.1487	236.1487
MGTS(18:4)	494.3482	18:4	236.1486	236.1486
MGTS(18:3)	496.3638	18:3	236.1486	236.1486
MGTS(18:2)	498.3795	18:2	236.1487	236.1487
MGTS(18:1)	500.3951	18:1	236.1486	236.1486
MGTS(20:5)	520.3638	20:5	236.1486	236.1486
MGTS(20:4)	522.3795	-	-	-
DGTS identified as [M+H]$^+$				
DGTS(28:1)	654.5309	16:1-12:0	236.1485	*418.3154-472.3618*
		14:1-14:0		428.3355-*444.3309*
DGTS(28:0)	656.5465	14:0/14:0	236.1486	*446.3463-446.3463*
		12:0-16:0		*474.3774-418.3154*
DGTS(30:3)	678.5309	16:3-.14:0	236.1485	*446.3450-468.3301*
DGTS(30:2)	680.5465	16:2-14:0	236.1487	*446.3462-470.3463*
		16:1-14:1		*444.3302-472.3641*
DGTS(30:1)	682.5622	16:1-14:0	236.1486	*446.3462-472.3620*
DGTS(32:5)	702.5309	20:5-12:0	236.1484	*418.3154-520.3611*
DGTS(32:4)	704.5465	18:4-14:0	236.1485	*446.3461-494.3476*
		-		-
DGTS(32:3)	706.5622	-		-
DGTS(32:2)	708.5778	16:1/16:1	236.1487	*472.3621-472.3621*
		18:2-14:0		*446.3460-498.3794*
		16:2-16:0		456.3674-*470.3474*
DGTS(32:1)	710.5935	16:1-16:0	236.1486	*474.3772-472.3618*
		18:1-14:0		*446.3458-500.3927*
DGTS(33:2)	722.5935	17:1-16:1	236.1489	454.3521-468.3701
		18:2-15:0		*460.3626-498.3768*

		17:2-16:0		*474.3812--*
DGTS(33:1)	724.6091	-	-	-
DGTS(34:6)	728.5465	20:5-14:1	236.1486	426.3190-*520.3622*
DGTS(34:5)	730.5622	20:5-14:0	236.1487	*446.3461-520.3624*
DGTS(34:4)	732.5778	20:4-14:0	236.1487	*446.3463*-504.3660
DGTS(34:3)	734.5935	20:3-14:0	236.1487	*446.3460*-506.3816
		16:1-18:2		*498.3785-472.3623*
		18:3-16:0		*474.3793--*
DGTS(34:2)	736.6091	18:2-16:0	236.1486	*474.3771-498.3775*
		18:1-16:1		*472.3621-482.3820*
		20:2-14:0		*446.3477--*
DGTS(34:1)	738.6248	20:1-14:0	236.149	*446.3468--*
		16:0-18:1		*500.3923--*
DGTS(36:8)	752.5465	-	-	-
DGTS(36:7)	754.5622	16:2-20:5	236.1487	*520.3622-470.3463*
DGTS(36:6)	756.5778	16:1-20:5	236.1486	*520.3620-472.3618*
DGTS(36:5)	758.5935	20:5-16:0	236.1489	*474.3782*-502.3524
DGTS(36:4)	760.6091	-	-	-
				-
DGTS(36:3)	762.6248	20:3-16:0	236.1485	*474.3778*-506.3833
		20:2-16:1		*472.3636-526.4072*
		18:2-18:1		*500.3916*-480.3686
DGTS(36:2)	764.6404	-	-	-
DGTS (37:5)	772.6091	20:5-17:0	236.1486	*488.3931-520.3621*
		20:4-17:1		*486.3768*-504.3666
DGTS(38:10)	776.5465	18:5-20:5	236.1486	*520.3611-492.3304*
DGTS(38:9)	778.5622	18:4-20:5	236.1485	*520.3645-494.3467*
DGTS(38:8)	780.5778	-	-	-
DGTS(38:7)	782.5935	20:5-18:2	236.1487	*498.3776-520.3626*
		18:3-20:4		504.3665--
DGTS(38:6)	784.6091	-	-	-
				-
DGTS(38:5)	786.6248	20:4-18:1	236.1486	*500.3924*-504.3695
		20:5-18:0		*502.4101--*
DGTS(40:10)	804.5778	20:5/20:5	236.1486	*520.3620-520.3620*
DGTS(40:9)	806.5935	-	-	-
Cer identified as $[M+H]^+$				
Cer(d32:2)	508.473	d18:1/14:1	264.2679, 282.2783	-
Cer(d32:1)	510.4886	-	-	-
MGMG identified as $[M+NH_4]^+$				
MGMG(16:0)	510.3642	-	-	-
DGMG identified as $[M+NH_4]^+$				
DGMG(14:0)	644.3857	-	-	-
DGMG(16:1)	670.4014	-	-	-
DGMG(16:0)	672.417	16:0	-	313.273

DGMG(20:5)	718.4014	-	-	-
MGDG identified as [M+NH$_4$]$^+$				
MGDG(30:1)	718.5464	-	-	-
MGDG(32:5)	738.5156	-	-	-
MGDG(32:2)	744.5626	-	-	-
		-		-
MGDG(32:1)	746.5777	-	-	-
		-		-
MGDG(34:5)	766.5469	14:0-20:5	-	285.2425--
		16:1-18:4		311.2577--
MGDG(34:2)	772.5933	-	-	-
		-		-
MGDG(34:1)	774.609	-	-	-
MGDG(36:6)	792.5625	16:1-20:5	-	311.2573--
MGDG(36:5)	794.5782	-	-	-
		-		-
MGDG(38:7)	818.5782	-	-	-
		-		-
MGDG(40:10)	840.5626	-	-	-
MGDG(40:9)	842.5782	-	-	-
MGDG(40:8)	844.5939	-	-	-
DGDG identified as [M+NH$_4$]$^+$				
DGDG(30:1)	880.5997	14:0-16:1	521.455	285.2416-311.2570
DGDG(30:0)	882.6154	-	-	-
DGDG(32:5)	900.5684	-	-	-
DGDG(32:3)	904.5997	-	-	-
DGDG(32:2)	906.6154	16:1/16:1	547.4705	311.2571-311.2571
		14:0-18:2		285.2410-337.2719
		16:0-16:2		313.2724--
DGDG(32:1)	908.631	16:0-16:1	549.486	313.2726-311.2570
		-		-
DGDG(34:5)	928.5997	14:0-20:5	569.4551	285.2423-359.2565
DGDG(34:3)	932.631	-	-	-
		-		-
DGDG(34:2)	934.6467	-	-	-
		-		-
		-		-
DGDG(34:1)	936.6623	-	-	-
		-		-
DGDG(35:5)	942.6154	15:0-20:5	-	299.2568-359.2566
DGDG(36:7)	952.5997	-	-	-
DGDG(36:6)	954.6154	16:1-20:5	595.4728	311.2572-359.2564
DGDG(36:5)	956.631	-	-	-
		-		-
DGDG(38:7)	980.631	18:2-20:5	-	337.2721--

DGDG(38:6)	982.6467	-	-	-
DGDG(40:10)	1002.6154	20:5/20:5	-	359.2566-359.2566

Abbreviations: NO, *N. oceanica*; Out, grown outdoors; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

Table S5.9. Assignment of polar lipids identified in the total lipid extracts of *N. oceanica* (NO) grown indoors (In) was confirmed by the presence of fragment ions and neutral losses of the polar heads and acyl side chains, as detailed in [33,35]. Briefly, the fatty acyl composition of PC, LPC, PG, LPG, PE, LPE, PI, and SQDG classes were assigned by the RCOO$^-$ fragment ions (in negative ion mode), DGTS and MGTS classes by the deduction by the losses of fatty acyl chains as acid (RCOOH), represented without italic and keto (R=C=O), represented in italic (in positive ion mode), DGDG, MGDG, DGMG and MGMG classes by the presence of product ions corresponding to each fatty acyl group as an acylium ion plus 74 (RCO + 74) (in positive ion mode).

***Nannochloropsis oceanica* - Indoor**				
Lipid species (C:N)	**Calculated *m/z***	**Fatty acyl chains (C:N)** **in MS/MS of the NO-In**	**Polar head *m/z* and neutral loss** **in MS/MS of the NO-In**	**Fatty acyl chains *m/z*** **in MS/MS of the NO-In**
LPC identified as [M+H]$^+$				
LPC(14:0)	468.309	14:0	184.0728	227.2006
LPC(16:3)	490.2934	-	184.0735	-
LPC(16:2)	492.309	16:2	184.073	251.2014
LPC(16:1)	494.3247	16:1	184.073	253.2169
LPC(16:0)	496.3403	16:0	184.073	255.2327
LPC(17:0)	510.356	-	-	-
LPC(17:1)	508.3403	17:1	184.0729	267.2327
LPC(17:2)	506.3247	17:2	184.0728	265.2169
LPC(18:5)	514.2934	-	-	-
LPC(18:4)	516.309	-	184.0729	-
LPC(18:3)	518.3247	18:3	184.073	277.217
LPC(18:2)	520.3403	18:2	184.0731	279.2326
LPC(18:1)	522.356	18:1	184.073	281.2482
LPC(20:5)	542.3247	20:5	184.073	301.2171
LPC(20:4)	544.3403	20:4	184.073	303.2325
LPC(20:3)	546.356	-	-	-
LPC(20:1)	550.3873	20:1	184.073	309.2798
LPC(20:0)	552.4029	-	184.0727	-
LPC(22:6)	568.3403	-	-	-
LPC(22:5)	570.356	-	184.0731	-
PC identified as [M+H]$^+$				

PC(28:1)	676.4917	-	184.0729	-
PC(30:3)	700.4917	-	184.072	-
PC(30:1)	704.523	14:0-16:1	184.073	227.2011-253.2169
		18:1-12:0		281.2487-199.1694
PC(31:2)	716.523	-	184.0731	-
		-		-
		-		-
PC(31:1)	718.5387	16:1-15:0	184.073	253.2169-241.2169
		16:0-15:1		255.2329-239.2013
		-		-
PC(32:5)	724.4917	-	-	-
PC(32:4)	726.5074	-	184.0729	-
		-		-
		-		-
PC(32:3)	728.523	16:2-16:1	184.073	251.2012-253.2169
		-		-
		-		-
PC(32:2)	730.5387	16:1/16:1	184.073	253.2170-253.2170
		16:2-16:0		251.2011-255.2327
		18:2-14:0		279.2326-227.2010
		18:1-14:1		281.2482-225.1853
PC(32:1)	732.5543	16:1-16:0	184.073	253.2168-255.2326
		18:1-14:0		281.2482-227.2009
PC(33:3)	742.5387	17:2-16:1	184.073	265.2170-253.2170
		17:1-16:2		267.2324-251.2004
		-		-
PC(33:2)	744.5543	17:1-16:1	184.073	267.2327-253.2169
		17:2-16:0		265.2173-255.2317
		18:1-15:1		281.2473-239.2005
		18:2-15:0		279.2330-241.2167
PC(34:7)	748.4917	-	-	-
PC(34:6)	750.5074	-	184.0731	-
PC(34:5)	752.523	14:0-20:5	184.073	227.2017-301.2181
		-		-
		-		-
		-		-
		-		-
PC(34:4)	754.5387	18:3-16:1	184.073	277.2168-253.2169
		18:2-16:2		279.2332-251.2009
		-		-
		-		-
		-		-
PC(34:3)	756.5543	18:1-16:2	184.073	281.2482-251.2012
		18:2-16:1		279.2325-253.2166
		18:3-16:0		277.2170-255.2325
		-		-

PC(34:2)	758.57	18:1-16:1	184.073	281.2484-253.2170
		18:2-16:0		279.2327-255.2325
PC(34:1)	760.5856	18:1-16:0	184.0727	281.2482-255.2325
		16:1-18:0		253.2170-283.2539
PC(35:4)	768.5543	18:2-17:2	184.0729	279.2328-265.2174
		18:3-17:1		277.2171-267.2342
PC(35:3)	770.57	18:1-17:2	184.073	281.2482-265.2169
		18:2-17:1		279.2330-267.2337
PC(35:2)	772.5856	18:1-17:1	184.073	281.2482-267.2325
		-		-
PC(35:1)	774.6013	-	184.073	-
		-		-
PC(37:7)	790.5387	-	184.0729	-
PC(37:6)	792.5543	17:1-20:5	184.073	267.2321-301.2169
		17:2-20:4		265.2182-303.2324
PC(36:8)	774.5074	-	184.0731	-
PC(36:7)	776.523	16:2-20:5	184.0729	251.2010-301.2175
		-		-
PC(36:6)	778.5387	20:5-16:1	184.073	301.2171-253.2170
		-		-
		-		-
		-		-
PC(36:5)	780.5543	-	184.073	-
		-		-
		-		-
PC(36:4)	782.57	18:2/18:2	184.073	279.2326-279.2326
		18:1-18:3		281.2483-277.2170
		20:4-16:0		303.2326-255.2326
		20:3-16:1		305.2484-253.2169
PC(36:4(OH))	798.5649	-	184.0731	-
PC(36:3)	784.5856	18:1-18:2	184.073	281.2483-279.2327
		20:2-16:1		307.2633-253.2171
		18:3-18:0		277.2168-283.2547
		20:3-16:0		305.2477-255.2327
PC(36:2)	786.6013	18:1/18:1	184.073	281.2483-281.2483
		18:2-18:0		279.2327-283.2552
		-		-
		-		-
PC(37:5)	794.57	17:1-20:4	184.073	267.2322-303.2325
		-	-	-
		-	-	-
		-	-	-
PC(37:4)	796.5856	-	184.0728	-
PC(37:3)	798.6013	-	184.0729	-
PC(37:2)	800.6169	-	184.073	-

PC(38:10)	798.5074	-	184.0731	-
PC(38:9)	800.523	-	184.0729	-
PC(38:8)	802.5387	-	184.073	-
		-		-
PC(38:7)	804.5543	18:2-20:5	184.0731	279.2327-301.2173
		18:3-20:4		277.2169-303.2332
PC(38:6)	806.57	18:1-20:5	184.0731	281.2484-301.2171
		18:2-20:4		279.2328-303.2326
		20:3-18:3		305.2483-277.2145
PC(38:5)	808.5856	18:1-20:4	184.073	281.2484-303.2327
		20:5-18:0		301.2171-283.2549
		18:2-20:3		279.2326-305.2423
PC(38:3)	812.6169	18:1-20:2	184.073	281.2485-307.2637
		20:3-18:0		305.2476-283.2545
		-		-
PC(38:2)	814.6326	18:2-20:0	184.073	279.2327-311.2955
		20:1-18:1		309.2798-281.2478
PC(38:1)	816.6482	18:1-20:0	184.073	281.2483-311.2950
PC(40:11)	824.523	-	184.073	-
PC(40:10)	826.5387	-	184.0729	-
PC(40:9)	828.5543	20:5-20:4	184.073	301.2171-303.2326
PC(40:8)	830.57	-	184.073	-
PC(40:5)	836.6169	20:1-20:4	184.0728	309.2798-303.2343
LPE identified as [M+H]$^+$				
LPE(14:0)	426.2621	-	285.241	-
LPE(16:3)	448.2464	-	-	-
LPE(16:2)	450.2621	16:2	309.2407	251.2013
LPE(16:1)	452.2777	16:1	311.2572	253.217
LPE(16:0)	454.2934	-	313.2728	-
LPE(18:4)	474.2621	-	333.2408	-
LPE(18:3)	476.2777	18:3	335.257	277.217
LPE(18:2)	478.2934	18:2	337.2727	279.2328
LPE(18:1)	480.309	18:1	339.2884	281.2484
LPE(20:5)	500.2777	20:5	359.2567	301.2171
LPE(20:4)	502.2934	20:4	361.2723	303.2329
LPE(20:3)	504.309	-	363.2877	-
LPE(20:2)	506.3247	20:2	365.3043	307.264
PE identified as [M+H]$^+$				
PE(30:3)	658.4448	-	517.4215	-
PE(30:1)	662.4761	14:0-16:1	521.4555	227.2009-253.2165
		15:1-15:0		239.2018-241.2171
		-		-
PE(30:0)	664.4917	-	-	-
		-		-
PE(32:4)	684.4604	-	-	-

PE(32:3)	686.4761	-	-	-
PE(32:2)	688.4917	16:1/16:1	547.4708	253.2169-253.2169
		16:0-16:2		255.2325-251.2060
PE(32:1)	690.5074	17:1-15:0	549.4858	267.2332-241.2173
		14:0-18:1		227.2021-281.2479
		16:0-16:1		255.2327-253.2169
PE(34:6)	708.4604	-	-	-
PE(34:5)	710.4761	-	569.4553	-
PE(34:4)	712.4917	-	571.47	-
		-		-
PE(34:3)	714.5074	18:2-16:1	573.4861	279.2325-253.2174
		-		-
		-		-
		-		-
PE(34:2)	716.523	18:1-16:1	575.5025	281.2481-253.2173
		18:2-16:0		279.2319-255.2349
		-		-
PE(36:8)	732.4604	-	-	-
PE(36:7)	734.4761	-	-	-
PE(36:6)	736.4917	16:1-20:5	595.4708	253.2168-301.2172
		16:2-20:4		251.2012-303.2322
PE(36:5)	738.5074	16:1-20:4	597.4857	253.2171-303.2325
		16:0-20:5		255.2320-301.2163
		-		-
PE(36:4)	740.523	-	599.4981	-
		-		-
PE(36:3)	742.5387	18:1-18:2	-	281.2481-279.2334
		16:0-20:3		255.2328-305.2489
		-		-
PE(36:2)	744.5543	-	-	-
PE(38:9)	758.4761	-	617.4521	-
PE(38:8)	760.4917	-	619.4681	-
		-		-
PE(38:7)	762.5074	18:2-20:5	621.4863	279.2327-301.2169
		-		277.2169-303.2324
		-		-
PE(38:6)	764.523	18:1-20:5	623.5009	281.2482-301.2171
		18:2-20:4		279.2326-303.2324
PE(38:5)	766.5387	18:1-20:4	625.5163	281.2484-303.2325
PE(40:10)	784.4917	20:5/20:5	643.4686	301.2170-301.2170
PE(40:9)	786.5074	20:4-20:5	645.4834	303.2326-301.2168
PE(40:8)	788.523	20:4/20:4	647.4984	303.2327-303.2327
		20:3-20:5		305.2483-301.2175
PE(40:7)	790.5387	20:5-20:2	-	301.2170-307.2647
		20:3-20:4		305.2481-303.2328
PE(40:1)	802.6326	-	-	-

		LPG identified as [M−H]$^-$		
LPG(16:0)	483.2723	-	-	-
		PG identified as [M−H]$^-$		
PG(30:1)	691.455	-	-	-
PG(31:0)	707.4863	-	-	-
PG(31:1)	705.4707	-	-	-
		-		-
PG(32:1)	719.4863	14:0-18:1	152.9946, 171.0052, 227.0320	227.2014-281.2478
		16:0-16:1		255.2325-253.2169
PG(32:2)	717.4707	-		-
		-		-
PG(33:1)	733.502	-		-
PG(34:1)	747.5176	16:0-18:1	152.9946, 171.0055	255.2325-281.2482
PG(34:2)	745.502	16:0-18:2	152.9944, 171.0058	255.2326-279.2328
		18:1-16:1		281.2483-253.2173
PG(34:5)	739.455	-		-
PG(35:5)	753.4707	15:0-20:5	152.9950, 171.0055	241.2169-301.2166
PG(36:2)	773.5333	-		-
PG(36:5)	767.4863	-		-
		-		-
PG(36:5(OH))	783.4812	(16:0-OH)-20:5	152.9945, 171.0057, 227.0337	271.2279-301.2171, 255.2329, 253.2173
PG(36:6)	765.4707	-	-	-
		-		-
		PI identified as [M−H]$^-$		
PI(28:0)	753.4554	-	-	-
PI(30:1)	779.4711	16:1-14:0	152.9946, 241.0114	253.2169-227.2010
PI(32:1)	807.5024	14:0-18:1	152.9946-241.0113	227.2010-281.2479
	807.5024	16:1-16:0		253.2168-255.2325
PI(32:2)	805.4867	14:0-18:2	152.9951, 241.0114	227.2011-279.2332
	805.4867	16:1-16:1		253.2169-253.2169
	805.4867	16:0-16:2		255.2328-251.2020
PI(33:1)	821.518	-	-	-
	821.518	-		-
	821.518	-		-
PI(34:1)	835.5337	16:0-18:1	152.9946, 241.0115	255.2328-281.2484
	835.5337			-
PI(34:2)	833.518	16:1-18:1	152.9946, 241.0114	253.2168-281.2484
	833.518	18:2-16:0		279.2326-255.2327
PI(34:3)	831.5024	-		-
	831.5024	-		-
PI(34:5)	827.4711	14:0-20:5	152.9946, 241.0115	227.2010-301.2172
PI(36:6)	853.4867	-	-	-
PI(40:10)	901.4867	-	-	-

		PI-Cer identified as [M−H]$^-$		
PI-Cer(d18:1/14:0)	750.4921	d18:1/14:0	241.0115, 259.0222	-
PI-Cer(d18:1/14:1)	748.4765	d18:1/14:1	241.0115, 259.0224	-
		SQDG identified as [M−H]$^-$		
SQDG(28:0)	737.451	-	-	-
SQDG(30:0)	765.4823	16:0-14:0	225.0069	255.2332-227.2020
SQDG(30:1)	763.4666	14:0-16:1	225.0069	227.2012-253.2170
SQDG(31:1)	777.4823	16:1-15:0	225.0069	253.2181-241.2173
SQDG(32:1)	791.4979	16:1-16:0	225.0069	253.2182-255.2313
SQDG(32:2)	789.4823	16:1/16:1	225.007	253.2168-253.2168
SQDG(32:3)	787.4666	-	225.007	-
SQDG(34:1)	819.5292	16:0-18:1	225.0067	255.2341-281.2481
SQDG(34:2)	817.5136	16:0-18:2	225.0069	255.2326-279.2332
SQDG(36:5)	839.4979	-	225.007	-
		MGTS identified as [M+H]$^+$		
MGTS(14:1)	444.3325	14:1	236.1484	236.1484
MGTS(14:0)	446.3482	14:0	236.1487	236.1487
MGTS(15:0)	460.3638	15:0	236.1488	236.1488
MGTS(16:4)	466.3169	16:4	236.1493	236.1493
MGTS(16:3)	468.3325	16:3	236.1487	236.1487
MGTS(16:2)	470.3482	-	-	-
MGTS(16:1)	472.3638	16:1	236.1487	236.1487
MGTS(16:0)	474.3795	-	-	-
MGTS (17:1)	486.3795	-	-	-
MGTS (17:2)	484.3638	17:2	236.1487	236.1487
MGTS(18:5)	492.3325	18:5	236.1486	236.1486
MGTS(18:4)	494.3482	18:4	236.1487	236.1487
MGTS(18:3)	496.3638	18:3	236.1486	236.1486
MGTS(18:2)	498.3795	18:2	236.1487	236.1487
MGTS(18:1)	500.3951	18:1	236.1486	236.1486
MGTS(20:5)	520.3638	20:5	236.1487	236.1487
MGTS(20:4)	522.3795	20:4	236.1487	236.1487
		DGTS identified as [M+H]$^+$		
DGTS(28:1)	654.5309	-	-	-
		-	-	-
DGTS(28:0)	656.5465	14:0-14:0	236.1486	*446.3464-446.3464*
		12:0-16:0		*474.3775--*
DGTS(30:3)	678.5309	-	-	-
DGTS(30:2)	680.5465	16:2-14:0	236.1485	*446.3462-470.3466*
		16:1-14:1		*444.3312-472.3637*
DGTS(30:1)	682.5622	16:1-14:0	236.1486	*446.3462-472.3617*
DGTS(32:5)	702.5309	20:5-12:0	236.1486	*418.3143--*
DGTS(32:4)	704.5465	18:4-14:0	236.1486	*446.3450*-476.3349
		-		-
DGTS(32:3)	706.5622	-		-

DGTS(32:2)	708.5778	18:2-14:0	236.1486	*446.3462-498.3782*
		16:1/16:1		*472.3620-472.3620*
		16:2-16:0		*474.3774-470.3461*
DGTS(32:1)	710.5935	16:1-16:0	236.1486	*474.3775-472.3618*
		18:1-14:0		*446.3462-500.3918*
DGTS(33:2)	722.5935	17:2-16:0	236.1487	*474.3776-484.3618*
		18:2-15:0		*460.3617*-480.3672
		17:1-16:1		*472.3618-486.3800*
DGTS(33:1)	724.6091	-	-	-
DGTS(34:6)	728.5465	-	-	-
DGTS(34:5)	730.5622	20:5-14:0	236.1487	*446.3464-520.3621*
DGTS(34:4)	732.5778	20:4-14:0	236.1489	*446.3458--*
DGTS(34:3)	734.5935	18:3-16:0	236.1488	*474.3778*-478.3520
		16:1-18:2		*498.3774-472.3623*
		20:3-14:0		*446.3470--*
DGTS(34:2)	736.6091	18:2-16:0	236.1486	*474.3776-498.3780*
		16:1-18:1		*500.3932-472.3619*
		-		-
DGTS(34:1)	738.6248	20:1-14:0	236.1487	*446.3462*--
		18:1-16:0		*474.3777*-482.3821
DGTS(36:8)	752.5465	-	-	-
DGTS(36:7)	754.5622	16:2-20:5	236.1487	*520.3625-470.3463*
DGTS(36:6)	756.5778	16:1-20:5	236.1487	*520.3624-472.3620*
DGTS(36:5)	758.5935	20:5-16:0	236.1489	*474.3776-520.3624*
DGTS(36:4)	760.6091	20:4-16:0	236.1487	*474.3777-522.3774*
		18:2/18:2		*498.3795-498.3795*
DGTS(36:3)	762.6248	-	-	-
		-		-
		-		-
DGTS(36:2)	764.6404	18:1/18:1	236.1487	*500.3932-500.3932*
DGTS (37:5)	772.6091	-	-	-
		-		-
DGTS(38:10)	776.5465	-	-	-
DGTS(38:9)	778.5622	18:4-20:5	236.1485	*520.3622-494.3489*
DGTS(38:8)	780.5778	18:3-20:5	236.1486	*520.3620-496.3621*
DGTS(38:7)	782.5935	18:2-20:5	236.1487	*520.3618-498.3776*
		18:3-20:4		*522.3763-496.3661*
DGTS(38:6)	784.6091	-	-	-
				-
DGTS(38:5)	786.6248	-	-	-
				-
DGTS(40:10)	804.5778	20:5/20:5	236.1487	*520.3620-520.3620*
DGTS(40:9)	806.5935	20:4-20:5	236.1486	*520.3622-522.3759*
Cer identified as [M+H]$^+$				
Cer(d32:2)	508.473	-	-	-

Cer(d32:1)	510.4886	d18:1/14:0	264.2679, 282.2783	-
MGMG identified as [M+NH$_4$]$^+$				
MGMG(16:0)	510.3642	16:0	-	313.2728
DGMG identified as [M+NH$_4$]$^+$				
DGMG(14:0)	644.3857	-	-	-
DGMG(16:1)	670.4014	-	-	-
DGMG(16:0)	672.417	-	-	-
DGMG(20:5)	718.4014	-	-	-
MGDG identified as [M+NH$_4$]$^+$				
MGDG(30:1)	718.5464	-	-	-
MGDG(32:5)	738.5156	12:0-20:5	-	257.2105--
MGDG(32:2)	744.5626	16:1-16:1	547.4748	311.2567-311.2567
		16:0-16:2		313.2744--
MGDG(32:1)	746.5777	16:0-16:1	549.4861	313.2728-311.2572
		14:0-18:1		285.2422-339.2901
MGDG(34:5)	766.5469	14:0-20:5	569.4579	285.2423-359.2575
		16:1-18:4		311.2594--
MGDG(34:2)	772.5933	16:0-18:2	575.5031	313.2734-337.2735
		16:1-18:1		311.2569--
MGDG(34:1)	774.609	16:0-18:1	577.5195	313.2727-339.2885
MGDG(36:6)	792.5625	16:1-20:5	-	311.2570-359.2568
MGDG(36:5)	794.5782	16:0-20:5	597.4901	313.2728-359.2575
		16:1-20:4		311.2581--
MGDG(38:7)	818.5782	18:2-20:5	-	337.2726--
		16:1-22:6		311.2573--
MGDG(40:10)	840.5626	20:5/20:5	-	359.2567-359.2567
MGDG(40:9)	842.5782	20:4-20:5	-	361.2705-359.2575
MGDG(40:8)	844.5939	20:4/20:4	-	361.2729-361.2729
DGDG identified as [M+NH$_4$]$^+$				
DGDG(30:1)	880.5997	14:0-16:1	521.4561	285.2413-311.2573
DGDG(30:0)	882.6154	14:0-16:0	523.4719	285.2430-313.2725
DGDG(32:5)	900.5684	-	-	-
DGDG(32:3)	904.5997	16:1/16:2	545.454	311.2569-309.2407
DGDG(32:2)	906.6154	16:1-16:1	547.4711	311.2571-311.2571
		16:0-16:2		313.2726-309.2414
		14:0-18:2		285.2415-337.2724
DGDG(32:1)	908.631	16:0-16:1	549.4861	313.2727-311.2571
		18:1-14:0		339.2899--
DGDG(34:5)	928.5997	14:0-20:5	569.4579	285.2424-359.2565
DGDG(34:3)	932.631	16:1-18:2	573.4874	311.2567-337.2731
		16:0-18:3		313.2717-335.2581
DGDG(34:2)	934.6467	16:0-18:2	575.5018	313.2728-337.2728
		16:1-18:1		311.2573-339.2886
		14:0-20:2		285.2412--
DGDG(34:1)	936.6623	-	-	-

		-		-
DGDG(35:5)	942.6154	-	-	-
DGDG(36:7)	952.5997	16:2-20:5	-	309.2417--
DGDG(36:6)	954.6154	16:1-20:5	595.4727	311.2571-359.2568
DGDG(36:5)	956.631	16:0-20:5	597.4849	313.2727-359.2566
		16:1-20:4		311.2572--
DGDG(38:7)	980.631	18:2-20:5	-	337.2728-359.2567
DGDG(38:6)	982.6467	-	-	-
DGDG(40:10)	1002.6154	20:5/20:5	-	359.2587-359.2587

Abbreviations: NO, *N. oceanica*; In, grown indoors;; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

Table S5.10. Assignment of polar lipids identified in the total lipid extracts of *N. limnetica* (NL) grown outdoors (Out) was confirmed by the presence of fragment ions and neutral losses of the polar heads and acyl side chains, as detailed in [33,35]. Briefly, the fatty acyl composition of PC, LPC, PG, LPG, PE, LPE, PI, and SQDG classes were assigned by the RCOO$^-$ fragment ions (in negative ion mode), DGTS and MGTS classes by the deduction by the losses of fatty acyl chains as acid (RCOOH), represented without italic and keto (R=C=O), represented in italic (in positive ion mode), DGDG, MGDG, DGMG and MGMG classes by the presence of product ions corresponding to each fatty acyl group as an acylium ion plus 74 (RCO + 74) (in positive ion mode).

Nannochloropsis limnetica - Outdoor				
Lipid species (C:N)	**Calculated *m/z***	**Fatty acyl chains (C:N)**	**Polar head *m/z* and neutral loss**	**Fatty acyl chains *m/z***
		in MS/MS of the NL-Out	**in MS/MS of the NL-Out**	**in MS/MS of the NL-Out**
LPC identified as [M+H]$^+$				
LPC(14:0)	468.309	14:0	184.0729	227.201
LPC(16:3)	490.2934	16:3	184.073	249.187
LPC(16:2)	492.309	16:2	184.073	251.2012
LPC(16:1)	494.3247	16:1	184.073	253.2168
LPC(16:0)	496.3403	16:0	184.0731	255.2329
LPC(17:0)	510.356	-	-	-
LPC(17:1)	508.3403	17:1	184.0729	267.2327
LPC(17:2)	506.3247	17:2	184.073	265.2169
LPC(18:5)	514.2934	-	184.0731	-
LPC(18:4)	516.309	18:4	184.073	275.2015
LPC(18:3)	518.3247	18:3	184.0731	277.217
LPC(18:2)	520.3403	18:2	184.073	279.2327
LPC(18:1)	522.356	18:1	184.073	281.2483
LPC(20:5)	542.3247	20:5	184.073	301..2170
LPC(20:4)	544.3403	-	-	-

LPC(20:3)	546.356	20:3	184.0731	305.2482
LPC(20:1)	550.3873	-	-	-
LPC(20:0)	552.4029	20:0	184.073	311.2955
LPC(22:6)	568.3403	-	184.0722	-
LPC(22:5)	570.356	22:5	184.073	329.2487
PC identified as [M+H]$^+$				
PC(28:1)	676.4917	-	184.0731	-
PC(30:3)	700.4917	-	184.0728	-
PC(30:1)	704.523	16:1-14:0	184.073	253.2169-227.2009
		14:1-16:0		225.1842-255.2322
PC(31:2)	716.523	16:1-15:1	184.073	253.2166-239.2011
		16:2-15:0		251.2015-241.2170
		17:2-14:0		265.2172-227.2018
PC(31:1)	718.5387	16:1-15:0	184.073	253.2170-241.2166
		16:0-15:1		255.2327-239.2016
		17:1-14:0		267.2331-227.2010
PC(32:5)	724.4917	16:1-16:4	184.073	253.2177-247.1699
PC(32:4)	726.5074	16:1-16:3	184.0731	253.2168-249.1855
		16:0-16:4		255.2329-247.1699
		16:2/16:2		251.2011-251.2005
PC(32:3)	728.523	18:3-14:0	184.0731	277.2167-227.2010
		16:2-16:1		251.2012-253.2168
		16:3-16:0		249.1856-255.2323
PC(32:2)	730.5387	18:2-14:0	184.0731	279.2327-227.2010
		16:2:16:0		251.2014-255.2325
		16:1/16:1		253.2170-253.2170
		-		-
PC(32:1)	732.5543	16:1-16:0	184.0728	253.2170-255.2327
		18:1-14:0		281.2481-227.2011
PC(33:3)	742.5387	17:2-16:1	184.0731	265.2171-253.2170
		17:1-16:2		267.2332-251.2012
		18:3-15:0		277.2165-241.2164
PC(33:2)	744.5543	17:1-16:1	184.0731	267.2328-253.2169
		17:2-16:0		265.2180-255.2328
		18:2-15:0		279.2327-241.2169
		-		-
PC(34:7)	748.4917	-	184.0729	-
PC(34:6)	750.5074	-	184.0731	-
PC(34:5)	752.523	20:5-14:0	184.0731	301.2170-227.2011
		18:2-16:3		279.2326-249.1855
		18:5-16:0		273.1857-255.2327
		18:3-16:2		277.2165-251.2019
		18:4-16:1		275.2020-253.2173
PC(34:4)	754.5387	20:4-14:0	184.0731	303.2325-227.2010
		18:1-16:3		281.2483-249.1853
		18:2-16:2		279.2322-251.2012

		16:1-18:3		253.2169-277.2170
		-		-
PC(34:3)	756.5543	20:3-14:0	184.0731	305.2488-227.2010
		18:1-16:2		281.2483-251.2011
		18:3-16:0		277.2170-255.2327
		18:2-16:1		279.2327-253.2169
PC(34:2)	758.57	18:1-16:1	184.0731	281.2484-253.2170
		18:2-16:0		279.2328-255.2327
PC(34:1)	760.5856	18:1-16:0	184.0729	281.2484-255.2327
		16:1-18:0		253.2169-283.2545
PC(35:4)	768.5543	-	184.0731	-
		-		-
PC(35:3)	770.57	18:1-17:2	184.073	281.2492-265.2169
		-		-
PC(35:2)	772.5856	18:1-17:1	184.0731	281.2479-267.2334
		-		-
PC(35:1)	774.6013	18:1-17:0	184.073	281.2482-269.2478
		-		-
PC(37:7)	790.5387	17:2-20:5	184.073	265.2167-301.2165
PC(37:6)	792.5543	17:1-20:5	184.0728	267.2327-301.2169
		17:2-20:4		265.2175-303.2317
		21:5-16:1		315.2322-253.2168
PC(36:8)	774.5074	-	184.073	-
PC(36:7)	776.523	16:2-20:5	184.0731	251.2012-301.2169
		16:3-20:4		249.1862-303.2322
PC(36:6)	778.5387	20:5-16:1	184.0731	301.2169-253.2170
		20:4-16:2		303.2328-251.2016
		-		-
		-		-
PC(36:5)	780.5543	20:5-16:0	184.0731	301.2170-255.2326
		16:1-20:4		253.2169-303.2323
		-		-
PC(36:4)	782.57	-	184.073	-
		-		-
		-		-
		-		-
PC(36:4(OH))	798.5649	-	184.073	
PC(36:3)	784.5856	20:3-16:0	184.073	305.2483-255.2325
		16:1-20:2		253.2173-307.2601
		18:2-18:1		279.2327-281.2483
		-		-
PC(36:2)	786.6013	18:1/18:1	184.073	281.2482-281.2482
		-		-
		-		-
		-		-
PC(37:5)	794.57	20:4-17:1	184.0731	303.2331-267.2321

		20:5-17:0		301.2180-269.2482
		21:5-16:0		315.2324-255.2326
		16:1-21:4		253.2168-317.2483
PC(37:4)	796.5856	21:4-16:0	184.073	317.2472-255.2327
PC(37:3)	798.6013	-	184.073	-
PC(37:2)	800.6169	-	184.073	-
PC(38:10)	798.5074	-	184.073	-
PC(38:9)	800.523	20:5-18:4	184.073	301.2176-275.2018
PC(38:8)	802.5387	18:3-20:5	184.073	277.2168-301.2169
		18:4-20:4		275.2013-303.2333
PC(38:7)	804.5543	18:2-20:5	184.073	279.2326-301.2170
		18:3-20:4		277.2169-303.2325
PC(38:6)	806.57	18:1-20:5	184.073	281.2483-301.2170
		18:2-20:4		279.2327-303.2325
		18:3-20:3		277.2177-305.2479
PC(38:5)	808.5856	18:1-20:4	184.073	281.2488-303.2320
		-		-
		-		-
PC(38:3)	812.6169	18:1-20:2	184.073	281.2487-307.2650
		18:3-20:0		277.2179-311.2961
		20:3-18:0		305.2480- 283.2565
PC(38:2)	814.6326	18:2-20:0	184.073	279.2326-311.2948
		-		-
PC(38:1)	816.6482	18:1-20:0	184.073	281.2474-311.2965
PC(40:11)	824.523	-	184.073	-
PC(40:10)	826.5387	20:5/20:5	184.073	301.2171-301.2171
PC(40:9)	828.5543	20:4-20:5	184.0731	303.2336-301.2197
PC(40:8)	830.57	-	184.073	-
PC(40:5)	836.6169	20:0-20:5	184.0731	311.2951-301.2171
LPE identified as [M+H]$^+$				
LPE(14:0)	426.2621	14:0	285.2421	227.2009
LPE(16:3)	448.2464	-	307.225	-
LPE(16:2)	450.2621	16:2	309.2417	251.2012
LPE(16:1)	452.2777	16:1	311.2571	253.2169
LPE(16:0)	454.2934	-	-	-
LPE(18:4)	474.2621	18:4	333.2408	275.2015
LPE(18:3)	476.2777	18:3	335.257	277.169
LPE(18:2)	478.2934	18:2	337.2727	279.2326
LPE(18:1)	480.309	18:1	339.2884	281.2483
LPE(20:5)	500.2777	20:5	359.2569	301.2169
LPE(20:4)	502.2934	20:4	361.2725	303.232
LPE(20:3)	504.309	-	363.2881	-
LPE(20:2)	506.3247	-	-	-
PE identified as [M+H]$^+$				
PE(30:3)	658.4448	-	517.4216	-

PE(30:1)	662.4761	16:1-14:0	521.4551	253.2169-227.2010
		16:0-14:1		255.2321-225.1853
		15:1-15:0		239.2013-241.2166
PE(30:0)	664.4917	14:0-16:0	-	227.2009-255.2327
		-		-
PE(32:4)	684.4604	-	543.4365	-
PE(32:3)	686.4761	16:2-16:1	545.4551	251.2014-253.2168
PE(32:2)	688.4917	14:0-18:2	547.471	227.2014-279.2326
		16:1/16:1		253.2169-253.2169
PE(32:1)	690.5074	16:0-16:1	-	255.2327-253.2170
		-		-
		-		-
PE(34:6)	708.4604	-	-	-
PE(34:5)	710.4761	-	569.4527	-
PE(34:4)	712.4917	14:0-20:4	571.468	227.2009-303.2330
		18:3-16:1		277.2163-253.2166
PE(34:3)	714.5074	18:2-16:1	573.4859	279.2326-253.2168
		18:3-16:0		277.2164-255.2331
		18:1-16:2		281.2484-251.2012
		20:3-14:0		305.2480-227.2010
PE(34:2)	716.523	16:1-18:1	575.5019	253.2169-281.2483
		17:1-17:1		267.2334-267.2334
		18:2-16:0		279.2325-255.2325
PE(36:8)	732.4604	-	-	
PE(36:7)	734.4761	16:2-20:5	593.4545	251.2010-301.2168
PE(36:6)	736.4917	16:1-20:5	595.4705	253.2169-301.2172
		16:2-20:4		251.2010-303.2316
PE(36:5)	738.5074	16:1-20:4	597.4854	253.2169-303.2324
		16:0-20:5		255.2326-301.2171
		-		-
PE(36:4)	740.523	20:3-16:1	599.5006	305.2485-253.2166
		16:0-20:4		255.2332-303.2311
PE(36:3)	742.5387	18:2-18:1	601.5172	279.2326-281.2483
		20:3-16:0		305.2485-255.2327
		16:1-20:2		253.2169-307.2614
PE(36:2)	744.5543	18:1/18:1	603.5331	281.2483-281.2483
PE(38:9)	758.4761	-	617.4519	-
PE(38:8)	760.4917	18:3-20:5	619.468	277.2168-301.2178
		20:4-18:4		303.2289-275.3032
PE(38:7)	762.5074	18:2-20:5	621.4852	279.2327-301.2169
		18:3-20:4		277.2170-303.2328
		-		-
PE(38:6)	764.523	18:2-20:4	623.5002	279.2328-303.2327
		18:1-20:5		281.2483-301.2177
PE(38:5)	766.5387	18:1-20:4	625.516	281.2481-303.2323
PE(40:10)	784.4917	20:5/20:5	-	301.2170-301.2170

PE(40:9)	786.5074	-	645.4834	-
PE(40:8)	788.523	20:4/20:4	647.4997	303.2327-303.2327
		20:3-20:5		305.2484-301.2169
PE(40:7)	790.5387	20:3-20:4	649.5159	305.2483-303.2326
		20:5-20:2		301.2170-307.2634
PE(40:1)	802.6326	-	-	-
LPG identified as [M−H]$^-$				
LPG(16:0)	483.2723	-	-	-
PG identified as [M−H]$^-$				
PG(30:1)	691.455	14:0-16:1	152.9946, 171.0051, 227.0323	227.2010-253.2168
PG(31:0)	707.4863	15:0-16:0	152.9945, 171.0050	241.2167-255.2327
PG(31:1)	705.4707	-		-
		-		-
PG(32:1)	719.4863	16:0-16:1	152.9946, 171.0052	255.2325-253.2168
		14:0-18:1		227.2009-281.2477
PG(32:2)	717.4707	16:1/16:1	152.9943, 171.0061	253.2169-253.2169
		16:2-16:0		251.2015-255.2326
PG(33:1)	733.502	-		-
PG(34:1)	747.5176	-		-
PG(34:2)	745.502	16:1-18:1	152.9947, 171.0053, 227.0323	253.2168-281.2482
		16:0-18:2		255.2324-279.2323
PG(34:5)	739.455	14:0-20:5	152.9947, 171.0054, 227.0326	227.2010-301.2168
PG(35:5)	753.4707	15:0-20:5	152.9946, 171.0053, 227.0340	241.2169-301.2166
PG(36:2)	773.5333	18:1-18:1	152.9946, 171.0052, 227.0324	281.2484-281.2484
PG(36:5)	767.4863	16:0-20:5	152.9947, 171.0054, 227.0325	255.2325-301.2169
		-		-
PG(36:5(OH))	783.4812	(16:0-OH)-20:5	152.9947, 171.0053, 227.0323	271.2276-301.2169
PG(36:6)	765.4707	20:5-16:1	152.9946, 171.0053, 227.0318	301.2162-253.2169
		20:4-18:1		303.2325-281.2483
PI identified as [M−H]$^-$				
PI(28:0)	753.4554	-	-	-
PI(30:1)	779.4711	16:1-14:0	152.9946, 241.0114	253.2169-227.2010
PI(32:1)	807.5024	14:0-18:1	152.9946, 241.0113	227.2010-281.2479
	807.5024	16:1-16:0		253.2168-255.2325
PI(32:2)	805.4867	14:0-18:2	152.9951, 241.0114	227.2011-279.2332
	805.4867	16:1/16:1		253.2169-253.2169
	805.4867	16:0-16:2		255.2328-251.2020
PI(33:1)	821.518	-	-	-
	821.518	-		-
	821.518	-		-

PI(34:1)	835.5337	16:0-18:1	152.9946, 241.0115	255.2328-281.2484
	835.5337	-		-
PI(34:2)	833.518	16:1-18:1	152.9946, 241.0114	253.2168-281.2484
	833.518	18:2-16:0		279.2326-255.2327
PI(34:3)	831.5024	-	-	-
	831.5024	-		-
PI(34:5)	827.4711	14:0-20:5	152.9946, 241.0115	227.2010-301.2172
PI(36:6)	853.4867	-	-	-
PI(40:10)	901.4867	-	-	-
PI-Cer identified as [M−H]$^{-}$				
PI-Cer(d18:1/14:0)	750.4921	-	-	-
PI-Cer(d18:1/14:1)	748.4765	d18:1/14:1	241.0113, 259.0219	-
SQDG identified as [M−H]$^{-}$				
SQDG(28:0)	737.451	-		-
SQDG(30:0)	765.4823	16:0-14:0	225.0067	255.2332-227.2020
SQDG(30:1)	763.4666	14:0-16:1	225.0069	227.2012-253.2170
SQDG(31:1)	777.4823	16:1-15:0	225.0067	253.2173-241.2172
SQDG(32:1)	791.4979	16:1-16:0	225.0069	253.2182-255.2313
SQDG(32:2)	789.4823	16:1/16:1	225.0069	253.2168-253.2168
SQDG(32:3)	787.4666	-	225.007	-
SQDG(34:1)	819.5292	16:0-18:1	225.007	255.2341-281.2481
SQDG(34:2)	817.5136	16:0-18:2	225.0067	255.2326-279.2332
SQDG(36:5)	839.4979	-	225.0072	-
MGTS identified as [M+H]$^{+}$				
MGTS(14:1)	444.3325	14:1	236.1486	236.1486
MGTS(14:0)	446.3482	14:0	236.1488	236.1488
MGTS(15:0)	460.3638	15:0	236.1487	236.1487
MGTS(16:4)	466.3169	16:4	236.1493	236.1493
MGTS(16:3)	468.3325	16:3	236.1487	236.1487
MGTS(16:2)	470.3482	16:2	236.1487	236.1487
MGTS(16:1)	472.3638	16:1	236.1487	236.1487
MGTS(16:0)	474.3795	16:0	236.1488	236.1488
MGTS (17:1)	486.3795	17:1	236.1488	236.1488
MGTS (17:2)	484.3638	-	-	-
MGTS(18:5)	492.3325	18:5	236.1487	236.1487
MGTS(18:4)	494.3482	18:4	236.1485	236.1485
MGTS(18:3)	496.3638	18:3	236.1487	236.1487
MGTS(18:2)	498.3795	-	-	-
MGTS(18:1)	500.3951	18:1	236.1487	236.1487
MGTS(20:5)	520.3638	20:5	236.1488	236.1488
MGTS(20:4)	522.3795	-	-	-
DGTS identified as [M+H]$^{+}$				
DGTS(28:1)	654.5309	-	-	-
		-	-	-

DGTS(28:0)	656.5465	14:0/14:0	236.1487	*446.3463-446.3463*
		12:0-16:0		*474.3776--*
DGTS(30:3)	678.5309	16:3-.14:0	236.1485	*446.3442-468.3309*
DGTS(30:2)	680.5465	16:2-14:0	236.149	428.3364--
		-	-	-
DGTS(30:1)	682.5622	16:1-14:0	236.1487	*446.3464-472.3622*
DGTS(32:5)	702.5309	-	-	-
DGTS(32:4)	704.5465	18:4-14:0	236.1484	428.3365-*494.3472*
		-		-
DGTS(32:3)	706.5622	-		-
DGTS(32:2)	708.5778	16:1/16:1	236.1487	*472.3621-472.6321*
		18:2-14:0		*446.3464-498.3788*
		16:2-16:0		*474.3763--*
DGTS(32:1)	710.5935	16:1-16:0	236.1488	*474.3779-472.3625*
		18:1-14:0		*446.3474-500.3959*
DGTS(33:2)	722.5935	-	-	-
		-		-
		-		-
DGTS(33:1)	724.6091	-	-	-
DGTS(34:6)	728.5465	20:5-14:1	236.1488	*444.3311-520.3646*
DGTS(34:5)	730.5622	20:5-14:0	236.1487	*446.3463-520.3626*
DGTS(34:4)	732.5778	20:4-14:0	236.1487	*446.3464--*
DGTS(34:3)	734.5935	18:3-16:0	236.1485	*474.3782--*
		16:1-18:2		*498.3764-472.3621*
		-		
DGTS(34:2)	736.6091	18:2-16:0	236.1486	*474.3772-498.3796*
		16:1-18:1		*500.3939*-454.3559
		-		-
DGTS(34:1)	738.6248	-	236.1482	-
		16:0-18:1		*500.3934-474.3763*
DGTS(36:8)	752.5465	-	-	-
DGTS(36:7)	754.5622	-	-	-
DGTS(36:6)	756.5778	20:5-16:1	236.1487	*472.3625-520.3622*
DGTS(36:5)	758.5935	20:5-16:0	236.1488	*474.3779-520.3619*
DGTS(36:4)	760.6091	-	-	-
		-		-
DGTS(36:3)	762.6248	-	-	-
		-		-
		-		-
DGTS(36:2)	764.6404	-	-	-
DGTS (37:5)	772.6091	20:5-17:0	236.1487	*488.3938-520.3620*
		20:4-17:1		468.3690--
DGTS(38:10)	776.5465	18:5-20:5	236.1487	*520.3619-492.3333*
DGTS(38:9)	778.5622	18:4-20:5	236.1489	*520.3606-494.3464*
DGTS(38:8)	780.5778	20:5-18:3	236.1486	*496.3629-520.3624*
DGTS(38:7)	782.5935	-	-	-

		-		-
DGTS(38:6)	784.6091	20:5-18:1	236.1487	*500.3925-520.3613*
		20:4-18:2		*498.3768-522.3767*
DGTS(38:5)	786.6248	20:4-18:1	236.1487	*500.3928*-504.3661
		20:5-18:0		*502.4103-520.3629*
DGTS(40:10)	804.5778	20:5/20:5	236.1487	*520.3622-520.3622*
DGTS(40:9)	806.5935	-	-	-
Cer identified as $[M+H]^+$				
Cer(d32:2)	508.473	d18:1/14:1	264.2678, 282.2780	-
Cer(d32:1)	510.4886	-	-	-
MGMG identified as $[M+NH_4]^+$				
MGMG(16:0)	510.3642	-	-	-
DGMG identified as $[M+NH_4]^+$				
DGMG(14:0)	644.3857	-	-	-
DGMG(16:1)	670.4014	-	-	-
DGMG(16:0)	672.417	16:0	-	313.273
DGMG(20:5)	718.4014	-	-	-
MGDG identified as $[M+NH_4]^+$				
MGDG(30:1)	718.5464	-	-	-
MGDG(32:5)	738.5156	12:0-20:5	-	257.2106--
MGDG(32:2)	744.5626	-	-	-
		-		-
MGDG(32:1)	746.5777	-	-	-
		-		-
MGDG(34:5)	766.5469	14:0-20:5	569.457	285.2421-359.2557
		-	-	-
MGDG(34:2)	772.5933	-	-	-
		-		-
MGDG(34:1)	774.609	-	-	-
MGDG(36:6)	792.5625	16:1-20:5	-	311.2571-359.2563
MGDG(36:5)	794.5782	16:0-20:5	597.4837	313.2726-359.2571
		16:1-20:4		311.2562--
MGDG(38:7)	818.5782	18:2-20:5	-	337.2716--
		16:1-22:6		311.2576--
MGDG(40:10)	840.5626	20:5/20:5	-	359.2568-359.2568
MGDG(40:9)	842.5782	20:4-20:5	-	361.2700-359.2571
MGDG(40:8)	844.5939	20:4/20:4	-	361.2720-361.2720
DGDG identified as $[M+NH_4]^+$				
DGDG(30:1)	880.5997	-	-	-
DGDG(30:0)	882.6154	-	-	-
DGDG(32:5)	900.5684	-	-	-
DGDG(32:3)	904.5997	16:1-16:2	-	311.2555-313.2761
DGDG(32:2)	906.6154	16:1/16:1	547.4718	311.2571-311.2571
		16:0-16:2		313.2727-309.2411
		14:0-18:2		285.2397-337.2707

DGDG(32:1)	908.631	16:0-16:1	549.4861	313.2726-311.2571
		18:1-14:0		339.2885--
DGDG(34:5)	928.5997	14:0-20:5	569.455	285.2425-359.2568
DGDG(34:3)	932.631	-	-	-
		-	-	-
DGDG(34:2)	934.6467	-	-	-
		-		-
		-		-
DGDG(34:1)	936.6623	-	-	-
		-		-
DGDG(35:5)	942.6154	15:0-20:5	-	299.2571--
DGDG(36:7)	952.5997	-	-	-
DGDG(36:6)	954.6154	16:1-20:5	595.4736	311.2571-359.2571
DGDG(36:5)	956.631	16:0-20:5	597.4856	313.2727-359.2568
		16:1-20:4		311.2570--
DGDG(38:7)	980.631	-	-	-
DGDG(38:6)	982.6467	-	-	-
DGDG(40:10)	1002.6154	20:5/20:5	-	359.2573-359.2573

Abbreviations: NL, *N. limnetica*; Out, grown outdoors; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

Table S5.11. Assignment of polar lipids identified in the total lipid extracts of *N. limnetica* (NL) grown indoors (In) was confirmed by the presence of fragment ions and neutral losses of the polar heads and acyl side chains, as detailed in [33,35]. Briefly, the fatty acyl composition of PC, LPC, PG, LPG, PE, LPE, PI, and SQDG classes were assigned by the RCOO- fragment ions (in negative ion mode), DGTS and MGTS classes by the deduction by the losses of fatty acyl chains as acid (RCOOH), represented without italic and keto (R=C=O), represented in italic (in positive ion mode), DGDG, MGDG, DGMG and MGMG classes by the presence of product ions corresponding to each fatty acyl group as an acylium ion plus 74 (RCO + 74) (in positive ion mode).

***Nannochloropsis limnetica* - Indoor**				
Lipid species (C:N)	**Calculated *m/z***	**Fatty acyl chains (C:N) in MS/MS of the NL-In**	**Polar head *m/z* and neutral loss in MS/MS of the NL-In**	**Fatty acyl chains *m/z* in MS/MS of the NL-In**
LPC identified as $[M+H]^+$				
LPC(14:0)	468.309	14:0	184.0729	227.2009
LPC(16:3)	490.2934	-	184.0733	-
LPC(16:2)	492.309	16:2	184.0729	251.2013
LPC(16:1)	494.3247	16:1	184.0731	253.2169
LPC(16:0)	496.3403	16:0	184.0731	255.2327

LPC(17:0)	510.356	-	184.0727	-
LPC(17:1)	508.3403	17:1	184.073	267.2322
LPC(17:2)	506.3247	17:2	184.0731	265.2168
LPC(18:5)	514.2934	-	184.0728	-
LPC(18:4)	516.309	-	184.073	-
LPC(18:3)	518.3247	18:3	184.0731	277.2168
LPC(18:2)	520.3403	18:2	184.073	279.2326
LPC(18:1)	522.356	18:1	184.0731	281.2482
LPC(20:5)	542.3247	20:5	184.0731	301.217
LPC(20:4)	544.3403	20:4	184.0731	303.2325
LPC(20:3)	546.356	-	-	-
LPC(20:1)	550.3873	-	-	-
LPC(20:0)	552.4029	20:0	184.0733	311.2953
LPC(22:6)	568.3403	-	184.0727	-
LPC(22:5)	570.356	-	-	-
PC identified as [M+H]$^+$				
PC(28:1)	676.4917	-	184.0731	-
PC(30:3)	700.4917	-	-	-
PC(30:1)	704.523	14:0-16:0	184.073	227.2010-253.2170
		-		-
PC(31:2)	716.523	16:1-15:1	184.0729	253.2171-239.2015
		-		-
		-		-
PC(31:1)	718.5387	16:1-15:0	184.073	253.2168-241.2168
		17:1-14:0		267.2323-227.2009
		-		-
PC(32:5)	724.4917	-	184.0725	-
PC(32:4)	726.5074	16:3-16:1	184.073	249.1852-253.2165
		16:2-16:2		251.2005-251.2005
		-		-
PC(32:3)	728.523	16:2-16:1	184.0731	251.2013-253.2170
		16:3-16:0		249.1854-255.2330
		-		-
PC(32:2)	730.5387	16:1/16:1	184.0731	253.2170-253.2170
		16:2-16:0		251.2011-255.2330
		-		-
		-		-
PC(32:1)	732.5543	16:1-16:0	184.0731	253.2170-255.2327
		18:1-14:0		281.2483-227.2010
PC(33:3)	742.5387	16:1-17:2	184.073	253.2168-265.2172
		17:1-16:2		267.2331-251.2025
		-		-
PC(33:2)	744.5543	17:1-16:1	184.0731	267.2326-253.2168
		17:2-16:0		265.2169-255.2327
		18:2-15:0		279.2327-241.2166
		-		-

PC(34:7)	748.4917	-	-	-
PC(34:6)	750.5074	-	184.073	-
PC(34:5)	752.523	14:0-20:5	184.0731	227.2011-301.2177
		18:2-16:3		279.2329-249.1857
		18:3-16:2		277.2163-251.2013
		16:1-18:4		253.2162-275.2013
		-		-
PC(34:4)	754.5387	18:2-16:2	184.0731	279.2330-251.2010
		18:3-16:1		277.2173-253.2169
		-		-
		-		-
		-		-
PC(34:3)	756.5543	18:1-16:2	184.0731	281.2483-251.2012
		18:2-16:1		279.2328-253.2168
		18:3-16:0		277.2169-255.2327
		-		-
PC(34:2)	758.57	18:1-16:1	184.073	281.2483-253.2168
		18:2-16:0		279.2326-255.2325
PC(34:1)	760.5856	18:1-16:0	184.0727	281.2483-255.2327
		16:1-18:0		253.2165-283.2556
PC(35:4)	768.5543	18:2-17:2	184.0731	279.226-265.2170
		18:3-17:1		277.2171-267.2321
PC(35:3)	770.57	18:1-17:2	184.0731	281.2482-265.2169
		18:2-17:1		279.2325-267.2326
PC(35:2)	772.5856	18:2-17:0	184.0731	279.2323-269.2124
		18:1-17:1		281.2476-267.2326
PC(35:1)	774.6013	18:1-17:0	184.0731	281.2482-269.2482
		18:0-17:1		283.2596-267.2337
PC(37:7)	790.5387	20:5-17:2	184.073	301.2171-265.2172
PC(37:6)	792.5543	17:1-20:5	184.073	267.2327-301.2175
		17:2-20:4		265.2177-303.2343
		-		-
PC(36:8)	774.5074	-	184.073	-
PC(36:7)	776.523	16:2-20:5	184.073	251.2018-301.2167
		-		-
PC(36:6)	778.5387	16:1-20:5	184.0731	253.2169-301.2170
		16:2-20:4		251.2016-303.2333
		18:3-18:3		277.2176--
		18:2-18:4		279.2331-275.1995
PC(36:5)	780.5543	-	184.073	-
		-		-
		-		-
PC(36:4)	782.57	-	184.073	-
		-		-
		-		-
		-		-

PC(36:4(OH))	798.5649	18:2-18:2-OH	184.073	279.2336-295.2268
PC(36:3)	784.5856	18:1-18:2	184.073	281.2483-279.2327
		20:3-16:0		305.2481-255.2320
		20:2-16:1		307.2627-253.2181
		-		-
PC(36:2)	786.6013	18:1/18:1	184.073	281.2482-281.2482
		18:2-18:0		279.2327-283.2543
		16:2-20:0		251.2011-311.2951
		-		-
PC(37:5)	794.57	17:1-20:4	184.073	267.2328-303.2323
		-		-
		-		-
		-		-
PC(37:4)	796.5856	-	184.073	-
PC(37:3)	798.6013	-	184.073	-
PC(37:2)	800.6169	-	184.073	-
PC(38:10)	798.5074	-	184.073	-
PC(38:9)	800.523	-	184.073	-
PC(38:8)	802.5387	18:3-20:5	184.073	277.2171-301.2168
		-		-
PC(38:7)	804.5543	18:2-20:5	184.0731	279.2326-301.2171
		18:3-20:4		277.2168-303.2327
PC(38:6)	806.57	18:2-20:4	184.0731	279.2327-303.2326
		18:1-20:5		281.2483-301.2170
		-		-
PC(38:5)	808.5856	18:1-20:4	184.073	281.2484-303.2326
		18:2-20:3		279.2327-305.2435
		20:5-18:0		301.2160-283.2555
PC(38:3)	812.6169	18:3-20:0	184.073	277.2167-311.2943
		18:2-20:1		279.2326-309.2804
		18:1-20:2		281.2483-307.2635
PC(38:2)	814.6326	18:2-20:0	184.0731	279.2325-311.2953
		-		-
PC(38:1)	816.6482	18:1-20:0	184.0729	281.2482-311.2953
PC(40:11)	824.523	-	184.0731	-
PC(40:10)	826.5387	-	184.073	-
PC(40:9)	828.5543	20:4-20:5	184.073	303.2325-301.2168
PC(40:8)	830.57	-	184.073	-
PC(40:5)	836.6169	20:0-20:5	184.073	311.2948-301.2174
LPE identified as $[M+H]^+$				
LPE(14:0)	426.2621	14:0	285.2412	227.201
LPE(16:3)	448.2464	-	-	-
LPE(16:2)	450.2621	16:2	309.2417	251.2012
LPE(16:1)	452.2777	16:1	311.2571	253.2169
LPE(16:0)	454.2934	-	313.2723	-
LPE(18:4)	474.2621	18:4	333.241	275.2018

LPE(18:3)	476.2777	18:3	335.2566	277.217
LPE(18:2)	478.2934	18:2	337.2727	279.2325
LPE(18:1)	480.309	18:1	339.2884	281.2483
LPE(20:5)	500.2777	20:5	359.2568	301.2171
LPE(20:4)	502.2934	20:4	361.2722	303.2328
LPE(20:3)	504.309	-	363.2867	-
LPE(20:2)	506.3247	20:2	-	307.2617
PE identified as [M+H]$^+$				
PE(30:3)	658.4448	-	517.4216	-
PE(30:1)	662.4761	14:0-16:1	521.4561	227.2008-253.2171
		-		-
		-		-
PE(30:0)	664.4917	15:0-15:0	-	241.2167-241.2168
		14:0-16:0		227.2018-255.2327
PE(32:4)	684.4604	-	543.4431	-
PE(32:3)	686.4761	-	545.4572	-
PE(32:2)	688.4917	16:1/16:1	547.4709	253.2170-253.2170
		16:2-16:0		251.1996-255.2311
PE(32:1)	690.5074	16:0-16:1	549.4858	255.2327-253.2170
		14:0-18:1		227.2013-281.2486
		17:1-15:0		267.2330-241.2160
PE(34:6)	708.4604	-	-	-
PE(34:5)	710.4761	-	-	-
PE(34:4)	712.4917	-	571.4684	-
		-		-
PE(34:3)	714.5074	18:2-16:1	573.4851	279.2328-253.2175
		-		-
		-		-
		-		-
PE(34:2)	716.523	17:1-17:1	575.502	267.2336-267.2336
		16:1-18:1		253.2169-281.2483
		18:2-16:0		279.2333-255.2336
PE(36:8)	732.4604	-	-	-
PE(36:7)	734.4761	-	593.4529	-
PE(36:6)	736.4917	16:1-20:5	595.4697	253.2169-301.2169
		16:2-20:4		251.2013-303.2322
PE(36:5)	738.5074	-	-	-
		-		-
		-		-
PE(36:4)	740.523	16:0-20:4	599.5004	255.2314-303.2337
		-		-
PE(36:3)	742.5387	18:2-18:1	-	279.2325-281.2484
		16:1-20:2		253.2160-307.2650
		16:0-20:3		255.2324-305.2478
PE(36:2)	744.5543	18:1/18:1	-	281.2483-281.2483
PE(38:9)	758.4761	-	617.4523	-

PE(38:8)	760.4917	18:3-20:5	619.4679	277.2166-301.2166
		18:4-20:4		275.2014-303.2336
PE(38:7)	762.5074	-	621.485	-
		-		-
		-		-
PE(38:6)	764.523	18:2-20:4	623.5024	279.2328-303.2327
		18:1-20:5		281.2483-301.2177
PE(38:5)	766.5387	18:1-20:4	625.517	281.2484-303.2326
PE(40:10)	784.4917	20:5/20:5	643.4675	301.2164-301.2164
PE(40:9)	786.5074	20:4-20:5	645.484	303.2327-301.2165
PE(40:8)	788.523	20:4/20:4	647.4987	303.2325-303.2325
		20:3-20:5		305.2480-301.2174
PE(40:7)	790.5387	20:3-20:4	649.5148	305.2472-303.2322
		-		-
PE(40:1)	802.6326	-	-	-
LPG identified as $[M-H]^-$				
LPG(16:0)	483.2723	-	-	-
PG identified as $[M-H]^-$				
PG(30:1)	691.455	14:0-16:1	152.9941, 171.0063	227.2010-253.2171
PG(31:0)	707.4863	-	-	-
PG(31:1)	705.4707	-	-	-
		-		-
PG(32:1)	719.4863	16:0-16:1	152.9946, 171.0054, 227.0319	255.2327-253.2170
		14:0-18:1		227.2015-281.2480
PG(32:2)	717.4707	-	-	-
		-		-
PG(33:1)	733.502	-	-	-
PG(34:1)	747.5176	16:0-18:1	152.9946, 171.0052	255.2327-281.2484
PG(34:2)	745.502	16:0-18:2	152.9949, 171.0054	255.2326-279.2326
		16:1-18:1		253.2167-281.2483
PG(34:5)	739.455	14:0-20:5	152.9946, 171.0052, 227.0316	227.2010-301.2168
PG(35:5)	753.4707	-	-	-
PG(36:2)	773.5333	-	-	-
PG(36:5)	767.4863	16:0-20:5	152.9945, 171.0052, 227.0315	255.2326-301.2170
		-		-
PG(36:5(OH))	783.4812	(16:0-OH)-20:5	-	271.2278-301.2167
PG(36:6)	765.4707	-	-	-
		-		-
PI identified as $[M-H]^-$				
PI(28:0)	753.4554	-	-	-
PI(30:1)	779.4711	-	-	-
PI(32:1)	807.5024	16:1-16:0	152.9947, 241.0114	253.2169-255.2327
	807.5024	-		-

PI(32:2)	805.4867	16:1/16:1	152.9849, 241.0116	253.2169-253.2169
	805.4867	-		-
	805.4867	-		-
PI(33:1)	821.518	15:0-18:1	152.9948, 241.0114	241.2169-281.2477
	821.518	16:0-17:1		255.2327-267.2326
	821.518	16:1-17:0		253.2175-269.2421
PI(34:1)	835.5337	16:0-18:1	152.9946, 241.0114	255.2327-281.2482
	835.5337	-		-
PI(34:2)	833.518	16:1-18:1	152.9948, 241.0114	253.2169-281.2482
	833.518	18:2-16:0		279.2326-255.25327
PI(34:3)	831.5024	-	-	-
	831.5024	-		-
PI(34:5)	827.4711	-	-	-
PI(36:6)	853.4867	-	-	-
PI(40:10)	901.4867	-	-	-
PI-Cer identified as [M−H]$^-$				
PI-Cer(d18:1/14:0)	750.4921	-	-	-
PI-Cer(d18:1/14:1)	748.4765	d18:1/14:1	241.0114, 259.0219	-
SQDG identified as [M−H]$^-$				
SQDG(28:0)	737.451	14:0/14:0	225.0068	227.2010-227.2010
SQDG(30:0)	765.4823	16:0-14:0	225.0067	255.2332-227.2006
SQDG(30:1)	763.4666	14:0-16:1	225.0067	227.2005-253.2167
SQDG(31:1)	777.4823	16:1-15:0	225.0067	253.2170-241.2172
SQDG(32:1)	791.4979	-	225.0068	-
SQDG(32:2)	789.4823	16:1/16:1	225.0071	253.2163-253.2163
SQDG(32:3)	787.4666	16:1-16:2	225.0072	253.2170-251.2017
SQDG(34:1)	819.5292	18:1-16:0	225.0067	281.2484-255.2329
SQDG(34:2)	817.5136	-	225.0068	-
SQDG(36:5)	839.4979	-	225.0066	-
MGTS identified as [M+H]$^+$				
MGTS(14:1)	444.3325	-	-	-
MGTS(14:0)	446.3482	14:0	236.1486	236.1486
MGTS(15:0)	460.3638	15:0	236.1492	236.1492
MGTS(16:4)	466.3169	-	-	-
MGTS(16:3)	468.3325	16:3	236.1484	236.1484
MGTS(16:2)	470.3482	-	-	-
MGTS(16:1)	472.3638	16:1	236.1487	236.1487
MGTS(16:0)	474.3795	16:0	236.1488	236.1488
MGTS (17:1)	486.3795	-	-	-
MGTS (17:2)	484.3638	17:2	236.1487	236.1487
MGTS(18:5)	492.3325	18:5	236.1487	236.1487
MGTS(18:4)	494.3482	18:4	236.1487	236.1487
MGTS(18:3)	496.3638	18:3	236.1487	236.1487

MGTS(18:2)	498.3795	18:2	236.1488	236.1488
MGTS(18:1)	500.3951	18:1	236.1487	236.1487
MGTS(20:5)	520.3638	20:5	236.1486	236.1486
MGTS(20:4)	522.3795	20:4	236.1486	236.1486
		DGTS identified as [M+H]$^+$		
DGTS(28:1)	654.5309	-	-	-
		-	-	-
DGTS(28:0)	656.5465	12:0-16:0	236.1485	*474.3774--*
		14:0/14:0		*446.3464-446.3464*
DGTS(30:3)	678.5309	-	-	-
DGTS(30:2)	680.5465	16:2-14:0	236.1487	*446.3471-470.3500*
		-	-	-
DGTS(30:1)	682.5622	16:1-14:0	236.1487	*446.3465-472.3621*
DGTS(32:5)	702.5309	-	-	-
DGTS(32:4)	704.5465	18:4-14:0	236.1486	*446.3466-494.3463*
		20:4-12:0		*418.3189-522.3807*
DGTS(32:3)	706.5622	18:3-14:0	236.1482	*446.3466--*
DGTS(32:2)	708.5778	16:1/16:1	236.1487	*472.3621-472.3621*
		18:2-14:0		*446.3465-498.3790*
		16:2-16:0		*474.3774-470.3477*
DGTS(32:1)	710.5935	16:0-16:1	236.1487	*472.3625-474.3776*
		18:1-14:0		*446.3443-500.3925*
DGTS(33:2)	722.5935	17:1-16:1	236.1487	*472.3623--*
		-		-
		17:2-16:0		*474.3766-484.3652*
DGTS(33:1)	724.6091	-	-	-
DGTS(34:6)	728.5465	20:5-14:1	236.149	*444.3313--*
DGTS(34:5)	730.5622	20:5-14:0	236.1487	*446.3464-520.3624*
DGTS(34:4)	732.5778	20:4-14:0	236.1488	*446.3468-522.3778*
DGTS(34:3)	734.5935	18:3-16:0	236.1488	*474.3778--*
		16:1-18:2		*498.3784-472.3634*
		20:3-14:0		*446.3476--*
DGTS(34:2)	736.6091	18:2-16:0	236.1487	*474.3774-498.3788*
		16:1-18:1		*500.3925-472.3635*
		-		-
DGTS(34:1)	738.6248	-	-	-
		-		-
DGTS(36:8)	752.5465	-	-	-
DGTS(36:7)	754.5622	-	-	-
DGTS(36:6)	756.5778	20:5-16:1	236.1487	*472.3619-520.3625*
DGTS(36:5)	758.5935	20:5-16:0	236.1487	*474.3776-520.3627*
DGTS(36:4)	760.6091	-	-	-
		-		-
DGTS(36:3)	762.6248	-	-	-
		-		-
		-		-

DGTS(36:2)	764.6404	-	-	-
DGTS (37:5)	772.6091	-	-	-
		-	-	-
DGTS(38:10)	776.5465	18:5-20:5	236.1487	*520.3621-492.3311*
DGTS(38:9)	778.5622	-	-	-
DGTS(38:8)	780.5778	18:3-20:5	236.1483	*520.3647--*
DGTS(38:7)	782.5935	20:5-18:2	236.1487	*498.3779-520.3612*
		18:3-20:4		*522.3777--*
DGTS(38:6)	784.6091	20:5-18:1	236.1487	*500.3928-520.3625*
		20:4-18:2		*498.3776*-504.3671
DGTS(38:5)	786.6248	20:4-18:1	236.1487	*500.3932-522.3770*
		18:0-20:5		*520.3644--*
DGTS(40:10)	804.5778	20:5/20:5	236.1487	*520.3622-520.3622*
DGTS(40:9)	806.5935	20:5-20:4	236.1488	*522.3759-520.3613*
Cer identified as [M+H]$^+$				
Cer(d32:2)	508.473	d18:1/14:1	264.2679, 282.2784	-
Cer(d32:1)	510.4886	-	-	-
MGMG identified as $[M+NH_4]^+$				
MGMG(16:0)	510.3642	-	-	-
DGMG identified as $[M+NH_4]^+$				
DGMG(14:0)	644.3857	-	-	-
DGMG(16:1)	670.4014	-	-	-
DGMG(16:0)	672.417	16:0	-	313.2731
DGMG(20:5)	718.4014	-	-	-
MGDG identified as $[M+NH_4]^+$				
MGDG(30:1)	718.5464	14:0-16:1	521.4553	285.2416-311.2571
MGDG(32:5)	738.5156	12:0-20:5	-	257.2103-359.2565
MGDG(32:2)	744.5626	16:1/16:1	547.4714	311.2571-311.2571
		16:0-16:2		313.2736-309.2418
MGDG(32:1)	746.5777	16:0-16:1	549.4863	313.2727-311.2571
		14:0-18:1		285.2421-339.2888
MGDG(34:5)	766.5469	14:0-20:5	569.4538	285.2422-359.2568
		16:1-18:4		311.2575--
MGDG(34:2)	772.5933	-	-	-
		-		-
MGDG(34:1)	774.609	16:0-18:1	577.5172	313.2734-339.2877
MGDG(36:6)	792.5625	16:1-20:5	595.469	311.2570-359.2575
MGDG(36:5)	794.5782	16:0-20:5	597.4835	313.2727-359.2565
		16:1-20:4		311.2569--
MGDG(38:7)	818.5782	18:2-20:5	-	337.2733--
		16:1-22:6		311.2573--
MGDG(40:10)	840.5626	20:5/20:5	-	359.2568-359.2568
MGDG(40:9)	842.5782	20:4-20:5	-	361.2694-359.2564
MGDG(40:8)	844.5939	20:4/20:4	-	361.2727-361.2727
DGDG identified as $[M+NH_4]^+$				

DGDG(30:1)	880.5997	14:0-16:1	521.4578	285.2422-311.2565
DGDG(30:0)	882.6154	14:0-16:0	523.4695	285.2414-313.2718
DGDG(32:5)	900.5684	-	-	-
DGDG(32:3)	904.5997	16:1-16:2	-	311.2565--
DGDG(32:2)	906.6154	16:1/16:1	547.4711	311.2572-311.2572
		16:0-16:2		313.2729-309.2419
		14:0-18:2		285.2413-337.2729
DGDG(32:1)	908.631	16:0-16:1	549.486	313.2726-311.2570
		14:0-18:1		285.2429--
DGDG(34:5)	928.5997	14:0-20:5	569.4552	285.2424-359.2564
DGDG(34:3)	932.631	-	-	-
		-	-	-
DGDG(34:2)	934.6467	16:0-18:2	575.5021	313.2727-337.2728
		16:1-18:1		311.2570-339.2890
		14:0-20:2		285.2419--
DGDG(34:1)	936.6623	16:0-18:1	577.5178	313.2726-339.2890
		16:1-18:0		311.2593--
DGDG(35:5)	942.6154	-	-	-
DGDG(36:7)	952.5997	16:2-20:5	-	309.2425--
DGDG(36:6)	954.6154	16:1-20:5	595.472	311.2571-359.2574
DGDG(36:5)	956.631	16:0-20:5	597.4852	313.2727-359.2566
		16:1-20:4		311.2574--
DGDG(38:7)	980.631	-	-	-
DGDG(38:6)	982.6467	-	-	-
DGDG(40:10)	1002.6154	20:5/20:5	-	359.2567-359.2567

Abbreviations: NL, *N. limnetica*; In, grown indoors; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

Table S5.12. Results of Kruskal-Wallis test (FDR adjusted) for statistically significant differences of polar lipid species between the groups. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

variables	.y.	n	statistic	df	p	method	p.adj	p.adj.signif
Cer(d32:2)	value	20	17.8706	3	0.0004680	Kruskal-Wallis	0.0020045	**
DGDG(30:0)	value	20	16.7080	3	0.0008120	Kruskal-Wallis	0.0020045	**

DGDG(32:1)	value	20	17.3314	3	0.0006040	Kruskal-Wallis	0.0020045	**
DGDG(34:1)	value	20	17.8840	3	0.0004650	Kruskal-Wallis	0.0020045	**
DGDG(34:2)	value	20	17.4674	3	0.0005660	Kruskal-Wallis	0.0020045	**
DGDG(34:3)	value	20	17.9245	3	0.0004560	Kruskal-Wallis	0.0020045	**
DGDG(35:5)	value	20	16.5543	3	0.0008730	Kruskal-Wallis	0.0020045	**
DGDG(36:6)	value	20	16.5086	3	0.0008920	Kruskal-Wallis	0.0020045	**
DGDG(40:10)	value	20	17.5829	3	0.0005360	Kruskal-Wallis	0.0020045	**
DGTS(28:0)	value	20	17.3575	3	0.0005970	Kruskal-Wallis	0.0020045	**
DGTS(28:1)	value	20	18.1159	3	0.0004160	Kruskal-Wallis	0.0020045	**
DGTS(30:1)	value	20	17.8571	3	0.0004710	Kruskal-Wallis	0.0020045	**
DGTS(30:2)	value	20	17.9652	3	0.0004470	Kruskal-Wallis	0.0020045	**
DGTS(30:3)	value	20	17.2979	3	0.0006140	Kruskal-Wallis	0.0020045	**
DGTS(32:1)	value	20	16.4400	3	0.0009210	Kruskal-Wallis	0.0020045	**
DGTS(32:2)	value	20	17.5829	3	0.0005360	Kruskal-Wallis	0.0020045	**
DGTS(32:3)	value	20	17.2402	3	0.0006310	Kruskal-Wallis	0.0020045	**
DGTS(32:4)	value	20	17.8571	3	0.0004710	Kruskal-Wallis	0.0020045	**
DGTS(33:1)	value	20	16.2845	3	0.0009910	Kruskal-Wallis	0.0020045	**
DGTS(33:2)	value	20	16.7521	3	0.0007950	Kruskal-Wallis	0.0020045	**
DGTS(34:1)	value	20	17.8706	3	0.0004680	Kruskal-Wallis	0.0020045	**
DGTS(34:3)	value	20	16.2236	3	0.0010200	Kruskal-Wallis	0.0020045	**
DGTS(34:6)	value	20	16.9737	3	0.0007160	Kruskal-Wallis	0.0020045	**
DGTS(36:2)	value	20	18.0843	3	0.0004230	Kruskal-Wallis	0.0020045	**
DGTS(36:3)	value	20	18.5065	3	0.0003460	Kruskal-Wallis	0.0020045	**
DGTS(36:4)	value	20	16.3829	3	0.0009460	Kruskal-Wallis	0.0020045	**
DGTS(38:9)	value	20	16.7595	3	0.0007920	Kruskal-Wallis	0.0020045	**
DGTS(40:9)	value	20	16.3029	3	0.0009830	Kruskal-Wallis	0.0020045	**
LPC(14:0)	value	20	17.7026	3	0.0005070	Kruskal-Wallis	0.0020045	**
LPC(16:1)	value	20	17.8571	3	0.0004710	Kruskal-Wallis	0.0020045	**
LPC(16:2)	value	20	17.6093	3	0.0005290	Kruskal-Wallis	0.0020045	**
LPC(16:3)	value	20	17.5051	3	0.0005560	Kruskal-Wallis	0.0020045	**
LPC(17:0)	value	20	18.7303	3	0.0003110	Kruskal-Wallis	0.0020045	**
LPC(18:1)	value	20	17.0343	3	0.0006950	Kruskal-Wallis	0.0020045	**
LPC(18:2)	value	20	17.1157	3	0.0006690	Kruskal-Wallis	0.0020045	**
LPC(18:3)	value	20	16.6565	3	0.0008320	Kruskal-Wallis	0.0020045	**
LPC(18:4)	value	20	17.8571	3	0.0004710	Kruskal-Wallis	0.0020045	**
LPC(18:5)	value	20	17.1000	3	0.0006740	Kruskal-Wallis	0.0020045	**
LPC(20:0)	value	20	16.6025	3	0.0008530	Kruskal-Wallis	0.0020045	**
LPC(20:1)	value	20	17.5926	3	0.0005340	Kruskal-Wallis	0.0020045	**
LPC(20:3)	value	20	17.8571	3	0.0004710	Kruskal-Wallis	0.0020045	**
LPC(20:5)	value	20	16.3695	3	0.0009520	Kruskal-Wallis	0.0020045	**
LPC(22:6)	value	20	18.0061	3	0.0004390	Kruskal-Wallis	0.0020045	**
LPE(14:0)	value	20	16.1801	3	0.0010400	Kruskal-Wallis	0.0020045	**
LPE(16:0)	value	20	17.8649	3	0.0004690	Kruskal-Wallis	0.0020045	**

LPE(16:2)	value	20	16.3948	3	0.0009410	Kruskal-Wallis	0.0020045	**
LPE(16:3)	value	20	16.3151	3	0.0009770	Kruskal-Wallis	0.0020045	**
LPE(18:3)	value	20	16.3805	3	0.0009470	Kruskal-Wallis	0.0020045	**
LPE(18:4)	value	20	16.4447	3	0.0009190	Kruskal-Wallis	0.0020045	**
LPE(20:5)	value	20	16.8971	3	0.0007420	Kruskal-Wallis	0.0020045	**
MGDG(30:1)	value	20	17.6095	3	0.0005290	Kruskal-Wallis	0.0020045	**
MGDG(32:1)	value	20	17.7438	3	0.0004970	Kruskal-Wallis	0.0020045	**
MGDG(32:2)	value	20	16.4791	3	0.0009040	Kruskal-Wallis	0.0020045	**
MGDG(32:5)	value	20	18.1298	3	0.0004140	Kruskal-Wallis	0.0020045	**
MGDG(34:1)	value	20	16.5732	3	0.0008650	Kruskal-Wallis	0.0020045	**
MGDG(34:2)	value	20	16.3230	3	0.0009740	Kruskal-Wallis	0.0020045	**
MGDG(34:5)	value	20	17.1157	3	0.0006690	Kruskal-Wallis	0.0020045	**
MGDG(36:5)	value	20	17.6093	3	0.0005290	Kruskal-Wallis	0.0020045	**
MGDG(36:6)	value	20	16.3274	3	0.0009720	Kruskal-Wallis	0.0020045	**
MGDG(38:7)	value	20	18.1298	3	0.0004140	Kruskal-Wallis	0.0020045	**
MGDG(40:10)	value	20	17.3314	3	0.0006040	Kruskal-Wallis	0.0020045	**
MGDG(40:8)	value	20	16.6797	3	0.0008220	Kruskal-Wallis	0.0020045	**
MGDG(40:9)	value	20	17.8706	3	0.0004680	Kruskal-Wallis	0.0020045	**
MGMG(16:0)	value	20	17.6492	3	0.0005200	Kruskal-Wallis	0.0020045	**
MGTS(14:0)	value	20	16.7143	3	0.0008090	Kruskal-Wallis	0.0020045	**
MGTS(15:0)	value	20	17.1544	3	0.0006570	Kruskal-Wallis	0.0020045	**
MGTS(16:0)	value	20	17.5829	3	0.0005360	Kruskal-Wallis	0.0020045	**
MGTS(16:1)	value	20	17.4503	3	0.0005710	Kruskal-Wallis	0.0020045	**
MGTS(16:2)	value	20	16.7269	3	0.0008040	Kruskal-Wallis	0.0020045	**
MGTS(16:3)	value	20	16.5543	3	0.0008730	Kruskal-Wallis	0.0020045	**
MGTS(16:4)	value	20	17.6879	3	0.0005100	Kruskal-Wallis	0.0020045	**
MGTS(17:1)	value	20	16.2652	3	0.0010000	Kruskal-Wallis	0.0020045	**
MGTS(17:2)	value	20	16.3839	3	0.0009460	Kruskal-Wallis	0.0020045	**
MGTS(18:1)	value	20	17.8571	3	0.0004710	Kruskal-Wallis	0.0020045	**
MGTS(18:3)	value	20	16.5667	3	0.0008680	Kruskal-Wallis	0.0020045	**
MGTS(18:4)	value	20	16.2457	3	0.0010100	Kruskal-Wallis	0.0020045	**
PC(28:1)	value	20	16.2112	3	0.0010300	Kruskal-Wallis	0.0020045	**
PC(30:1)	value	20	16.5201	3	0.0008870	Kruskal-Wallis	0.0020045	**
PC(31:2)	value	20	16.9663	3	0.0007180	Kruskal-Wallis	0.0020045	**
PC(32:2)	value	20	16.5543	3	0.0008730	Kruskal-Wallis	0.0020045	**
PC(32:3)	value	20	17.3314	3	0.0006040	Kruskal-Wallis	0.0020045	**
PC(32:4)	value	20	16.6942	3	0.0008170	Kruskal-Wallis	0.0020045	**
PC(32:5)	value	20	16.6131	3	0.0008490	Kruskal-Wallis	0.0020045	**
PC(33:3)	value	20	16.3397	3	0.0009660	Kruskal-Wallis	0.0020045	**
PC(34:1)	value	20	17.1029	3	0.0006730	Kruskal-Wallis	0.0020045	**
PC(34:3)	value	20	16.5771	3	0.0008630	Kruskal-Wallis	0.0020045	**
PC(34:5)	value	20	17.8571	3	0.0004710	Kruskal-Wallis	0.0020045	**
PC(34:6)	value	20	17.7974	3	0.0004840	Kruskal-Wallis	0.0020045	**

PC(34:7)	value	20	17.1604	3	0.0006550	Kruskal-Wallis	0.0020045	**
PC(35:2)	value	20	17.7305	3	0.0005000	Kruskal-Wallis	0.0020045	**
PC(36:2)	value	20	17.8571	3	0.0004710	Kruskal-Wallis	0.0020045	**
PC(36:6)	value	20	16.9242	3	0.0007330	Kruskal-Wallis	0.0020045	**
PC(36:8)	value	20	17.9245	3	0.0004560	Kruskal-Wallis	0.0020045	**
PC(37:3)	value	20	16.7286	3	0.0008040	Kruskal-Wallis	0.0020045	**
PC(38:1)	value	20	17.1804	3	0.0006490	Kruskal-Wallis	0.0020045	**
PC(38:10)	value	20	17.1763	3	0.0006500	Kruskal-Wallis	0.0020045	**
PC(38:2)	value	20	16.1802	3	0.0010400	Kruskal-Wallis	0.0020045	**
PC(38:9)	value	20	17.5829	3	0.0005360	Kruskal-Wallis	0.0020045	**
PC(40:10)	value	20	16.2236	3	0.0010200	Kruskal-Wallis	0.0020045	**
PC(40:5)	value	20	17.7429	3	0.0004970	Kruskal-Wallis	0.0020045	**
PC(40:8)	value	20	17.8571	3	0.0004710	Kruskal-Wallis	0.0020045	**
PE(30:0)	value	20	16.1863	3	0.0010400	Kruskal-Wallis	0.0020045	**
PE(32:2)	value	20	17.1157	3	0.0006690	Kruskal-Wallis	0.0020045	**
PE(32:3)	value	20	16.9973	3	0.0007080	Kruskal-Wallis	0.0020045	**
PE(34:2)	value	20	17.8706	3	0.0004680	Kruskal-Wallis	0.0020045	**
PE(34:3)	value	20	16.1867	3	0.0010400	Kruskal-Wallis	0.0020045	**
PE(34:5)	value	20	17.6759	3	0.0005130	Kruskal-Wallis	0.0020045	**
PE(36:2)	value	20	18.4681	3	0.0003520	Kruskal-Wallis	0.0020045	**
PE(36:3)	value	20	17.5856	3	0.0005350	Kruskal-Wallis	0.0020045	**
PE(36:8)	value	20	16.4864	3	0.0009010	Kruskal-Wallis	0.0020045	**
PE(38:5)	value	20	17.9245	3	0.0004560	Kruskal-Wallis	0.0020045	**
PE(38:6)	value	20	17.8571	3	0.0004710	Kruskal-Wallis	0.0020045	**
PG(31:0)	value	20	16.3081	3	0.0009800	Kruskal-Wallis	0.0020045	**
PG(34:5)	value	20	17.1929	3	0.0006450	Kruskal-Wallis	0.0020045	**
PG(36:5(OH))	value	20	16.9099	3	0.0007380	Kruskal-Wallis	0.0020045	**
PG(36:6)	value	20	16.4688	3	0.0009090	Kruskal-Wallis	0.0020045	**
PI-Cer(d18:1/14:0)	value	20	18.1298	3	0.0004140	Kruskal-Wallis	0.0020045	**
PI(28:0)	value	20	18.5306	3	0.0003420	Kruskal-Wallis	0.0020045	**
PI(30:1)	value	20	17.1551	3	0.0006570	Kruskal-Wallis	0.0020045	**
PI(34:3)	value	20	16.9824	3	0.0007130	Kruskal-Wallis	0.0020045	**
PI(34:5)	value	20	16.2206	3	0.0010200	Kruskal-Wallis	0.0020045	**
PI(36:6)	value	20	16.9954	3	0.0007080	Kruskal-Wallis	0.0020045	**
SQDG(31:1)	value	20	17.0571	3	0.0006880	Kruskal-Wallis	0.0020045	**
SQDG(34:2)	value	20	17.1544	3	0.0006570	Kruskal-Wallis	0.0020045	**
PC(40:11)	value	20	16.1659	3	0.0010500	Kruskal-Wallis	0.0020076	**
DGTS(32:5)	value	20	16.1435	3	0.0010600	Kruskal-Wallis	0.0020106	**
DGTS(36:6)	value	20	16.0864	3	0.0010900	Kruskal-Wallis	0.0020195	**
LPE(16:1)	value	20	16.0743	3	0.0010900	Kruskal-Wallis	0.0020195	**
PC(38:5)	value	20	16.0950	3	0.0010800	Kruskal-Wallis	0.0020195	**
PE(40:1)	value	20	16.0268	3	0.0011200	Kruskal-Wallis	0.0020591	**

PC(37:7)	value	20	16.0026	3	0.0011300	Kruskal-Wallis	0.0020616	**
DGDG(38:6)	value	20	15.8672	3	0.0012100	Kruskal-Wallis	0.0020626	**
DGDG(38:7)	value	20	15.8152	3	0.0012400	Kruskal-Wallis	0.0020626	**
DGMG(16:0)	value	20	15.8956	3	0.0011900	Kruskal-Wallis	0.0020626	**
DGTS(34:5)	value	20	15.7771	3	0.0012600	Kruskal-Wallis	0.0020626	**
DGTS(36:8)	value	20	15.8686	3	0.0012100	Kruskal-Wallis	0.0020626	**
MGTS(20:5)	value	20	15.9371	3	0.0011700	Kruskal-Wallis	0.0020626	**
PC(31:1)	value	20	15.8011	3	0.0012500	Kruskal-Wallis	0.0020626	**
PC(36:4(OH))	value	20	15.7802	3	0.0012600	Kruskal-Wallis	0.0020626	**
PC(36:5)	value	20	15.9371	3	0.0011700	Kruskal-Wallis	0.0020626	**
PC(36:7)	value	20	15.9068	3	0.0011800	Kruskal-Wallis	0.0020626	**
PC(38:3)	value	20	15.7732	3	0.0012600	Kruskal-Wallis	0.0020626	**
PE(34:4)	value	20	15.7701	3	0.0012600	Kruskal-Wallis	0.0020626	**
PG(32:2)	value	20	15.8904	3	0.0011900	Kruskal-Wallis	0.0020626	**
PG(36:2)	value	20	15.9182	3	0.0011800	Kruskal-Wallis	0.0020626	**
SQDG(36:5)	value	20	15.8563	3	0.0012100	Kruskal-Wallis	0.0020626	**
PG(31:1)	value	20	15.7312	3	0.0012900	Kruskal-Wallis	0.0020832	**
PI(40:10)	value	20	15.7259	3	0.0012900	Kruskal-Wallis	0.0020832	**
DGTS(34:4)	value	20	15.6975	3	0.0013100	Kruskal-Wallis	0.0020873	**
SQDG(30:1)	value	20	15.6889	3	0.0013100	Kruskal-Wallis	0.0020873	**
DGTS(37:5)	value	20	15.6574	3	0.0013300	Kruskal-Wallis	0.0021051	**
DGTS(40:10)	value	20	15.6171	3	0.0013600	Kruskal-Wallis	0.0021384	**
DGTS(34:2)	value	20	15.5460	3	0.0014000	Kruskal-Wallis	0.0021587	**
DGTS(38:6)	value	20	15.5714	3	0.0013900	Kruskal-Wallis	0.0021587	**
PE(36:4)	value	20	15.5468	3	0.0014000	Kruskal-Wallis	0.0021587	**
PE(40:8)	value	20	15.4229	3	0.0014900	Kruskal-Wallis	0.0022828	**
PC(38:8)	value	20	15.4114	3	0.0015000	Kruskal-Wallis	0.0022834	**
Cer(d32:1)	value	20	15.3573	3	0.0015400	Kruskal-Wallis	0.0023004	**
DGTS(38:5)	value	20	15.3660	3	0.0015300	Kruskal-Wallis	0.0023004	**
PE(36:5)	value	20	15.3576	3	0.0015400	Kruskal-Wallis	0.0023004	**
PC(34:2)	value	20	15.3200	3	0.0015600	Kruskal-Wallis	0.0023158	**
LPE(18:1)	value	20	15.2857	3	0.0015900	Kruskal-Wallis	0.0023457	**
PC(32:1)	value	20	15.2743	3	0.0016000	Kruskal-Wallis	0.0023460	**
PI(32:2)	value	20	15.2514	3	0.0016100	Kruskal-Wallis	0.0023463	**
PE(34:6)	value	20	15.2399	3	0.0016200	Kruskal-Wallis	0.0023465	**
PI(32:1)	value	20	15.2057	3	0.0016500	Kruskal-Wallis	0.0023756	**
DGMG(14:0)	value	20	15.1649	3	0.0016800	Kruskal-Wallis	0.0023900	**
SQDG(32:1)	value	20	15.1829	3	0.0016700	Kruskal-Wallis	0.0023900	**
DGDG(32:3)	value	20	15.0646	3	0.0017600	Kruskal-Wallis	0.0024456	**
LPC(20:4)	value	20	15.0799	3	0.0017500	Kruskal-Wallis	0.0024456	**
MGTS(18:2)	value	20	15.0686	3	0.0017600	Kruskal-Wallis	0.0024456	**
PC(38:6)	value	20	15.0686	3	0.0017600	Kruskal-Wallis	0.0024456	**
LPE(20:3)	value	20	15.0427	3	0.0017800	Kruskal-Wallis	0.0024591	**

MGTS(20:4)	value	20	14.9543	3	0.0018600	Kruskal-Wallis	0.0025115	**
PC(33:2)	value	20	14.9455	3	0.0018600	Kruskal-Wallis	0.0025115	**
PE(38:8)	value	20	14.9641	3	0.0018500	Kruskal-Wallis	0.0025115	**
PG(32:1)	value	20	14.9884	3	0.0018300	Kruskal-Wallis	0.0025115	**
DGTS(38:8)	value	20	14.9314	3	0.0018800	Kruskal-Wallis	0.0025243	**
DGTS(36:5)	value	20	14.8743	3	0.0019300	Kruskal-Wallis	0.0025626	**
PE(38:9)	value	20	14.8722	3	0.0019300	Kruskal-Wallis	0.0025626	**
PG(30:1)	value	20	14.8512	3	0.0019500	Kruskal-Wallis	0.0025749	**
DGTS(38:7)	value	20	14.8054	3	0.0019900	Kruskal-Wallis	0.0026132	**
DGDG(32:2)	value	20	14.7714	3	0.0020200	Kruskal-Wallis	0.0026381	**
PI(34:1)	value	20	14.7029	3	0.0020900	Kruskal-Wallis	0.0027147	**
PC(37:2)	value	20	14.6502	3	0.0021400	Kruskal-Wallis	0.0027646	**
PG(34:1)	value	20	14.6343	3	0.0021600	Kruskal-Wallis	0.0027755	**
MGTS(18:5)	value	20	14.5619	3	0.0022300	Kruskal-Wallis	0.0028349	**
PE(36:6)	value	20	14.5600	3	0.0022300	Kruskal-Wallis	0.0028349	**
LPC(22:5)	value	20	14.5380	3	0.0022600	Kruskal-Wallis	0.0028579	**
PI(34:2)	value	20	14.4514	3	0.0023500	Kruskal-Wallis	0.0029561	**
PC(36:3)	value	20	14.3600	3	0.0024500	Kruskal-Wallis	0.0030657	**
PC(35:3)	value	20	14.3108	3	0.0025100	Kruskal-Wallis	0.0031206	**
PC(37:5)	value	20	14.2993	3	0.0025200	Kruskal-Wallis	0.0031206	**
PE(30:1)	value	20	14.1459	3	0.0027100	Kruskal-Wallis	0.0033386	**
PE(36:7)	value	20	14.1046	3	0.0027700	Kruskal-Wallis	0.0033950	**
DGMG(16:1)	value	20	14.0680	3	0.0028100	Kruskal-Wallis	0.0034265	**
DGTS(36:7)	value	20	14.0468	3	0.0028400	Kruskal-Wallis	0.0034281	**
PG(33:1)	value	20	14.0528	3	0.0028300	Kruskal-Wallis	0.0034281	**
DGDG(36:5)	value	20	13.9029	3	0.0030400	Kruskal-Wallis	0.0036147	**
PC(34:4)	value	20	13.9029	3	0.0030400	Kruskal-Wallis	0.0036147	**
PC(36:4)	value	20	13.9029	3	0.0030400	Kruskal-Wallis	0.0036147	**
LPG(16:0)	value	20	13.8684	3	0.0030900	Kruskal-Wallis	0.0036560	**
PE(40:9)	value	20	13.6971	3	0.0033500	Kruskal-Wallis	0.0039441	**
PE(30:3)	value	20	13.6356	3	0.0034500	Kruskal-Wallis	0.0040419	**
LPE(18:2)	value	20	13.6131	3	0.0034800	Kruskal-Wallis	0.0040572	**
LPC(16:0)	value	20	13.5371	3	0.0036100	Kruskal-Wallis	0.0041282	**
PE(32:4)	value	20	13.5332	3	0.0036100	Kruskal-Wallis	0.0041282	**
PE(38:7)	value	20	13.5554	3	0.0035800	Kruskal-Wallis	0.0041282	**
PI-Cer(d18:1/14:1)	value	20	13.5371	3	0.0036100	Kruskal-Wallis	0.0041282	**
SQDG(32:2)	value	20	13.4987	3	0.0036700	Kruskal-Wallis	0.0041768	**
PE(40:10)	value	20	13.0212	3	0.0045900	Kruskal-Wallis	0.0051991	**
DGDG(36:7)	value	20	12.9200	3	0.0048100	Kruskal-Wallis	0.0054226	**
DGDG(30:1)	value	20	12.8708	3	0.0049200	Kruskal-Wallis	0.0055206	**
DGMG(20:5)	value	20	12.5665	3	0.0056700	Kruskal-Wallis	0.0063324	**
PC(30:3)	value	20	12.4462	3	0.0060000	Kruskal-Wallis	0.0066698	**

SQDG(28:0)	value	20	12.4147	3	0.0060900	Kruskal-Wallis	0.0067385	**
PC(35:4)	value	20	12.3822	3	0.0061800	Kruskal-Wallis	0.0068065	**
PE(32:1)	value	20	12.3083	3	0.0064000	Kruskal-Wallis	0.0070165	**
LPE(20:4)	value	20	12.1886	3	0.0067600	Kruskal-Wallis	0.0073774	**
LPC(17:2)	value	20	12.0711	3	0.0071400	Kruskal-Wallis	0.0077566	**
MGTS(14:1)	value	20	11.8776	3	0.0078100	Kruskal-Wallis	0.0084461	**
PG(36:5)	value	20	11.7771	3	0.0081900	Kruskal-Wallis	0.0088172	**
LPC(17:1)	value	20	11.7436	3	0.0083200	Kruskal-Wallis	0.0089170	**
DGTS(38:10)	value	20	11.7041	3	0.0084700	Kruskal-Wallis	0.0090372	**
PC(35:1)	value	20	11.6355	3	0.0087400	Kruskal-Wallis	0.0092838	**
SQDG(34:1)	value	20	11.4172	3	0.0096700	Kruskal-Wallis	0.0102262	*
LPE(20:2)	value	20	11.1664	3	0.0109000	Kruskal-Wallis	0.0114762	*
PE(40:7)	value	20	11.0312	3	0.0116000	Kruskal-Wallis	0.0121596	*
PC(37:6)	value	20	10.5843	3	0.0142000	Kruskal-Wallis	0.0148201	*
PG(35:5)	value	20	10.4929	3	0.0148000	Kruskal-Wallis	0.0153791	*
DGDG(32:5)	value	20	10.4083	3	0.0154000	Kruskal-Wallis	0.0159333	*
PI(33:1)	value	20	10.2594	3	0.0165000	Kruskal-Wallis	0.0169978	*
SQDG(32:3)	value	20	10.1499	3	0.0173000	Kruskal-Wallis	0.0177455	*
PC(38:7)	value	20	10.0704	3	0.0180000	Kruskal-Wallis	0.0183846	*
DGDG(34:5)	value	20	9.5143	3	0.0232000	Kruskal-Wallis	0.0235949	*
PC(37:4)	value	20	9.3908	3	0.0245000	Kruskal-Wallis	0.0248114	*
PG(34:2)	value	20	4.5720	3	0.2060000	Kruskal-Wallis	0.2077384	ns
PC(40:9)	value	20	3.6113	3	0.3070000	Kruskal-Wallis	0.3082899	ns
SQDG(30:0)	value	20	2.6686	3	0.4460000	Kruskal-Wallis	0.4460000	ns

Table S5.13. Results of Dunn's multiple comparison post-hoc test (FDR adjusted) of significant polar lipids (q values < 0.05) identified in the total lipid extracts of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

variable	.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
DGDG(34:1)	value	NO.In	NO.Out	5	5	-4.0119	0.0000602	0.0014128	**
DGDG(34:3)	value	NL.Out	NO.In	5	5	4.0165	0.0000591	0.0014128	**
DGDG(38:7)	value	NL.Out	NO.In	5	5	3.9584	0.0000754	0.0014128	**
DGDG(40:10)	value	NL.Out	NO.In	5	5	-3.9555	0.0000764	0.0014128	**
DGTS(28:1)	value	NL.In	NO.Out	5	5	4.0379	0.0000539	0.0014128	**
DGTS(30:1)	value	NL.In	NO.Out	5	5	4.0089	0.0000610	0.0014128	**
DGTS(30:2)	value	NL.In	NO.Out	5	5	4.0210	0.0000579	0.0014128	**
DGTS(32:2)	value	NL.In	NO.Out	5	5	3.9555	0.0000764	0.0014128	**
DGTS(32:4)	value	NL.In	NO.Out	5	5	4.0089	0.0000610	0.0014128	**

DGTS(34:1)	value	NO.In	NO.Out	5	5	-4.0104	0.0000606	0.0014128	**
DGTS(36:2)	value	NL.Out	NO.Out	5	5	-4.0502	0.0000512	0.0014128	**
DGTS(36:4)	value	NO.In	NO.Out	5	5	-3.9555	0.0000764	0.0014128	**
DGTS(38:9)	value	NO.In	NO.Out	5	5	3.9852	0.0000674	0.0014128	**
Cer(d32:2)	value	NL.In	NO.In	5	5	-4.0104	0.0000606	0.0014128	**
LPC(14:0)	value	NL.Out	NO.In	5	5	-3.9689	0.0000722	0.0014128	**
LPC(16:1)	value	NL.Out	NO.In	5	5	-4.0089	0.0000610	0.0014128	**
LPC(16:2)	value	NL.Out	NO.In	5	5	-4.0119	0.0000602	0.0014128	**
LPC(16:3)	value	NL.Out	NO.In	5	5	-3.9947	0.0000648	0.0014128	**
LPC(17:0)	value	NL.Out	NO.In	5	5	-4.1058	0.0000403	0.0014128	**
LPC(18:4)	value	NL.Out	NO.In	5	5	-4.0089	0.0000610	0.0014128	**
LPC(18:5)	value	NL.Out	NO.In	5	5	-4.0833	0.0000444	0.0014128	**
LPC(20:0)	value	NL.In	NO.In	5	5	-4.0456	0.0000522	0.0014128	**
LPC(20:3)	value	NL.In	NL.Out	5	5	4.0089	0.0000610	0.0014128	**
LPC(22:6)	value	NL.In	NL.Out	5	5	4.0256	0.0000568	0.0014128	**
LPE(16:0)	value	NL.Out	NO.Out	5	5	-4.0256	0.0000568	0.0014128	**
LPE(16:3)	value	NL.Out	NO.In	5	5	-4.0183	0.0000586	0.0014128	**
LPE(18:3)	value	NL.Out	NO.In	5	5	-3.9659	0.0000731	0.0014128	**
LPE(18:4)	value	NL.Out	NO.In	5	5	-3.9629	0.0000740	0.0014128	**
LPE(20:5)	value	NL.Out	NO.Out	5	5	-4.0089	0.0000610	0.0014128	**
MGDG(30:1)	value	NL.In	NO.Out	5	5	-4.0409	0.0000532	0.0014128	**
MGDG(32:1)	value	NL.In	NO.Out	5	5	-3.9852	0.0000674	0.0014128	**
MGDG(32:2)	value	NL.In	NO.Out	5	5	-4.0165	0.0000591	0.0014128	**
MGDG(32:5)	value	NL.In	NO.Out	5	5	-4.0394	0.0000536	0.0014128	**
MGDG(34:5)	value	NL.In	NO.Out	5	5	-4.0104	0.0000606	0.0014128	**
MGDG(36:5)	value	NL.In	NO.Out	5	5	-4.0119	0.0000602	0.0014128	**
MGDG(36:6)	value	NL.In	NO.Out	5	5	-4.0119	0.0000602	0.0014128	**
MGDG(38:7)	value	NL.In	NO.Out	5	5	-4.0394	0.0000536	0.0014128	**
MGDG(40:8)	value	NL.In	NO.Out	5	5	-4.0241	0.0000572	0.0014128	**
MGDG(40:9)	value	NL.In	NO.Out	5	5	-4.0104	0.0000606	0.0014128	**
MGMG(16:0)	value	NL.In	NO.Out	5	5	-3.9629	0.0000740	0.0014128	**
MGTS(15:0)	value	NL.In	NL.Out	5	5	4.0150	0.0000595	0.0014128	**
MGTS(16:0)	value	NL.Out	NO.Out	5	5	-4.0089	0.0000610	0.0014128	**
MGTS(16:1)	value	NL.In	NO.Out	5	5	3.9570	0.0000759	0.0014128	**
MGTS(16:4)	value	NL.In	NL.Out	5	5	4.1941	0.0000274	0.0014128	**
MGTS(17:1)	value	NL.In	NO.In	5	5	4.0241	0.0000572	0.0014128	**
MGTS(17:2)	value	NL.Out	NO.In	5	5	3.9765	0.0000700	0.0014128	**
MGTS(18:1)	value	NO.In	NO.Out	5	5	-4.0089	0.0000610	0.0014128	**
PC(30:1)	value	NO.In	NO.Out	5	5	4.0134	0.0000598	0.0014128	**
PC(32:2)	value	NO.In	NO.Out	5	5	4.0089	0.0000610	0.0014128	**
PC(32:3)	value	NO.In	NO.Out	5	5	4.0089	0.0000610	0.0014128	**
PC(33:3)	value	NO.In	NO.Out	5	5	4.0134	0.0000598	0.0014128	**
PC(34:1)	value	NO.In	NO.Out	5	5	-4.0089	0.0000610	0.0014128	**
PC(34:5)	value	NO.In	NO.Out	5	5	4.0089	0.0000610	0.0014128	**
PC(34:6)	value	NO.In	NO.Out	5	5	4.0180	0.0000587	0.0014128	**
PC(35:2)	value	NO.In	NO.Out	5	5	-3.9837	0.0000679	0.0014128	**
PC(36:2)	value	NO.In	NO.Out	5	5	-4.0089	0.0000610	0.0014128	**
PC(36:8)	value	NL.Out	NO.In	5	5	-4.0165	0.0000591	0.0014128	**
PC(38:10)	value	NL.Out	NO.In	5	5	-3.9719	0.0000713	0.0014128	**
PC(38:9)	value	NO.In	NO.Out	5	5	3.9555	0.0000764	0.0014128	**
PC(40:10)	value	NL.Out	NO.In	5	5	-4.0104	0.0000606	0.0014128	**

PC(40:11)	value	NL.Out	NO.In	5	5	-4.0003	0.0000633	0.0014128	**
PC(40:5)	value	NL.In	NO.Out	5	5	-3.9734	0.0000708	0.0014128	**
PC(40:8)	value	NL.Out	NO.In	5	5	4.0089	0.0000610	0.0014128	**
PE(34:2)	value	NL.Out	NO.In	5	5	-4.0104	0.0000606	0.0014128	**
PE(34:4)	value	NL.Out	NO.In	5	5	-3.9541	0.0000768	0.0014128	**
PE(34:5)	value	NL.Out	NO.In	5	5	-3.9659	0.0000731	0.0014128	**
PE(36:2)	value	NL.Out	NO.In	5	5	-4.0769	0.0000456	0.0014128	**
PE(36:3)	value	NL.Out	NO.Out	5	5	-4.1319	0.0000360	0.0014128	**
PE(38:5)	value	NO.In	NO.Out	5	5	-4.0165	0.0000591	0.0014128	**
PE(38:6)	value	NO.In	NO.Out	5	5	-4.0089	0.0000610	0.0014128	**
PE(40:1)	value	NL.In	NL.Out	5	5	3.9988	0.0000637	0.0014128	**
PG(36:5(OH))	value	NL.Out	NO.In	5	5	-4.0104	0.0000606	0.0014128	**
PI(30:1)	value	NL.In	NO.Out	5	5	4.0394	0.0000536	0.0014128	**
PI(34:3)	value	NL.In	NO.Out	5	5	4.0409	0.0000532	0.0014128	**
PI(36:6)	value	NL.In	NO.Out	5	5	4.0425	0.0000529	0.0014128	**
PI-Cer(d18:1/14:0)	value	NL.Out	NO.In	5	5	4.0394	0.0000536	0.0014128	**
SQDG(30:1)	value	NL.In	NO.Out	5	5	3.9570	0.0000759	0.0014128	**
PE(36:8)	value	NL.Out	NO.In	5	5	-3.9426	0.0000806	0.0014635	**
DGDG(34:2)	value	NO.In	NO.Out	5	5	-3.9302	0.0000849	0.0015212	**
DGDG(32:1)	value	NL.In	NO.Out	5	5	-3.9020	0.0000954	0.0015350	**
DGTS(28:0)	value	NL.In	NO.Out	5	5	3.9050	0.0000942	0.0015350	**
DGTS(34:2)	value	NL.Out	NO.In	5	5	3.9035	0.0000948	0.0015350	**
LPC(18:1)	value	NL.In	NO.Out	5	5	-3.9020	0.0000954	0.0015350	**
LPE(16:1)	value	NL.Out	NO.In	5	5	-3.9020	0.0000954	0.0015350	**
MGDG(40:10)	value	NL.In	NO.Out	5	5	-3.9020	0.0000954	0.0015350	**
PG(34:5)	value	NO.In	NO.Out	5	5	3.9035	0.0000948	0.0015350	**
PI(32:2)	value	NO.In	NO.Out	5	5	3.9020	0.0000954	0.0015350	**
SQDG(36:5)	value	NL.In	NL.Out	5	5	3.9079	0.0000931	0.0015350	**
DGMG(14:0)	value	NL.Out	NO.In	5	5	-3.8942	0.0000985	0.0015674	**
DGDG(36:6)	value	NL.In	NO.Out	5	5	3.8486	0.0001188	0.0015872	**
DGTS(30:3)	value	NL.In	NO.Out	5	5	3.8704	0.0001086	0.0015872	**
DGTS(32:3)	value	NL.In	NO.Out	5	5	3.8782	0.0001052	0.0015872	**
DGTS(36:5)	value	NL.Out	NO.Out	5	5	-3.8486	0.0001188	0.0015872	**
LPC(18:2)	value	NL.In	NO.Out	5	5	-3.8500	0.0001181	0.0015872	**
MGTS(18:4)	value	NL.In	NO.Out	5	5	3.8486	0.0001188	0.0015872	**
PC(36:6)	value	NO.In	NO.Out	5	5	3.8767	0.0001059	0.0015872	**
PC(37:3)	value	NO.In	NO.Out	5	5	-3.8631	0.0001120	0.0015872	**
PC(38:1)	value	NL.In	NO.Out	5	5	-3.8573	0.0001147	0.0015872	**
PE(30:0)	value	NL.Out	NO.Out	5	5	-3.8503	0.0001180	0.0015872	**
PE(32:2)	value	NL.Out	NO.In	5	5	-3.8500	0.0001181	0.0015872	**
PE(36:5)	value	NL.Out	NO.In	5	5	-3.8811	0.0001040	0.0015872	**
PE(38:8)	value	NL.Out	NO.In	5	5	-3.8616	0.0001126	0.0015872	**
PE(40:8)	value	NL.Out	NO.Out	5	5	3.8486	0.0001188	0.0015872	**
PG(36:2)	value	NL.Out	NO.Out	5	5	-3.8515	0.0001174	0.0015872	**
SQDG(31:1)	value	NL.In	NO.In	5	5	3.8486	0.0001188	0.0015872	**
SQDG(34:2)	value	NL.Out	NO.In	5	5	3.8544	0.0001160	0.0015872	**
DGDG(30:0)	value	NL.Out	NO.In	5	5	3.8247	0.0001309	0.0017326	**
DGTS(34:6)	value	NL.In	NO.Out	5	5	3.8037	0.0001426	0.0018690	**
PC(31:2)	value	NO.In	NO.Out	5	5	3.7948	0.0001478	0.0019117	**
PE(34:6)	value	NL.Out	NO.In	5	5	-3.7936	0.0001485	0.0019117	**

DGTS(33:1)	value	NL.In	NO.In	5	5	3.7812	0.0001561	0.0019910	**
LPE(16:2)	value	NL.Out	NO.In	5	5	-3.7788	0.0001576	0.0019925	**
PC(34:7)	value	NL.Out	NO.In	5	5	-3.7736	0.0001609	0.0020167	**
PE(32:3)	value	NL.Out	NO.In	5	5	-3.7648	0.0001667	0.0020704	**
DGTS(33:2)	value	NL.Out	NO.In	5	5	3.7459	0.0001798	0.0021393	**
DGTS(38:8)	value	NL.Out	NO.Out	5	5	-3.7417	0.0001828	0.0021393	**
LPE(18:1)	value	NO.In	NO.Out	5	5	-3.7417	0.0001828	0.0021393	**
MGTS(14:0)	value	NL.In	NL.Out	5	5	3.7417	0.0001828	0.0021393	**
MGTS(16:2)	value	NL.In	NO.In	5	5	3.7431	0.0001818	0.0021393	**
PC(34:3)	value	NL.In	NO.In	5	5	-3.7417	0.0001828	0.0021393	**
PC(36:5)	value	NL.Out	NO.In	5	5	-3.7417	0.0001828	0.0021393	**
DGTS(37:5)	value	NL.In	NL.Out	5	5	3.7276	0.0001933	0.0022440	**
PC(32:4)	value	NO.In	NO.Out	5	5	3.7219	0.0001977	0.0022760	**
LPC(18:3)	value	NL.Out	NO.In	5	5	-3.7177	0.0002010	0.0022956	**
DGDG(35:5)	value	NL.In	NO.Out	5	5	-3.6882	0.0002258	0.0024044	**
DGTS(32:1)	value	NL.Out	NO.In	5	5	3.6882	0.0002258	0.0024044	**
LPC(20:4)	value	NL.Out	NO.Out	5	5	-3.6896	0.0002246	0.0024044	**
MGTS(16:3)	value	NL.In	NL.Out	5	5	3.6882	0.0002258	0.0024044	**
MGTS(18:2)	value	NO.In	NO.Out	5	5	-3.6882	0.0002258	0.0024044	**
MGTS(18:3)	value	NL.Out	NO.Out	5	5	-3.6896	0.0002246	0.0024044	**
MGTS(18:5)	value	NL.In	NO.In	5	5	3.6910	0.0002234	0.0024044	**
PE(40:9)	value	NL.Out	NO.In	5	5	3.6882	0.0002258	0.0024044	**
PI(32:1)	value	NL.In	NO.In	5	5	3.6882	0.0002258	0.0024044	**
PC(37:7)	value	NL.Out	NO.In	5	5	-3.6739	0.0002388	0.0025052	**
PE(34:3)	value	NL.In	NL.Out	5	5	3.6739	0.0002388	0.0025052	**
DGTS(36:7)	value	NL.In	NO.Out	5	5	3.6642	0.0002481	0.0025798	**
LPE(14:0)	value	NL.Out	NO.In	5	5	-3.6608	0.0002514	0.0025798	**
PG(31:0)	value	NO.In	NO.Out	5	5	3.6624	0.0002499	0.0025798	**
PC(32:5)	value	NL.Out	NO.In	5	5	-3.6506	0.0002617	0.0026655	**
PC(36:4)	value	NO.In	NO.Out	5	5	-3.6348	0.0002782	0.0028143	**
PC(37:2)	value	NO.In	NO.Out	5	5	-3.6217	0.0002927	0.0029396	**
PE(36:7)	value	NL.Out	NO.In	5	5	-3.6135	0.0003021	0.0030128	**
Cer(d32:1)	value	NL.Out	NO.In	5	5	3.6094	0.0003069	0.0030181	**
LPC(20:5)	value	NL.Out	NO.Out	5	5	-3.6094	0.0003069	0.0030181	**
DGTS(32:5)	value	NL.In	NO.Out	5	5	3.6044	0.0003129	0.0030553	**
PE(36:6)	value	NL.Out	NO.In	5	5	-3.5894	0.0003314	0.0032144	**
DGTS(40:9)	value	NO.In	NO.Out	5	5	3.5813	0.0003419	0.0032274	**
LPC(16:0)	value	NL.Out	NO.In	5	5	-3.5813	0.0003419	0.0032274	**
MGTS(20:5)	value	NL.In	NL.Out	5	5	3.5813	0.0003419	0.0032274	**
PG(34:1)	value	NL.In	NO.Out	5	5	-3.5813	0.0003419	0.0032274	**
PE(30:3)	value	NL.In	NO.Out	5	5	-3.5694	0.0003578	0.0033557	**
PC(36:7)	value	NL.Out	NO.In	5	5	-3.5573	0.0003748	0.0034912	**
DGTS(34:3)	value	NL.Out	NO.In	5	5	3.5292	0.0004169	0.0038082	**
PE(40:10)	value	NO.In	NO.Out	5	5	-3.5292	0.0004169	0.0038082	**
PG(30:1)	value	NL.Out	NO.In	5	5	-3.5292	0.0004169	0.0038082	**
DGTS(36:3)	value	NL.In	NO.Out	5	5	3.5125	0.0004439	0.0039042	**
DGTS(36:3)	value	NL.Out	NO.Out	5	5	3.5125	0.0004439	0.0039042	**
DGTS(36:3)	value	NO.In	NO.Out	5	5	3.5125	0.0004439	0.0039042	**
PI(28:0)	value	NL.In	NO.Out	5	5	3.5148	0.0004401	0.0039042	**
PI(28:0)	value	NL.Out	NO.Out	5	5	3.5148	0.0004401	0.0039042	**
PI(28:0)	value	NO.In	NO.Out	5	5	3.5148	0.0004401	0.0039042	**

DGDG(38:6)	value	NL.Out	NO.In	5	5	3.5051	0.0004565	0.0039899	**
PC(33:2)	value	NO.In	NO.Out	5	5	3.5024	0.0004610	0.0040049	**
LPC(20:1)	value	NL.In	NO.In	5	5	3.4953	0.0004735	0.0040566	**
LPC(20:1)	value	NO.In	NO.Out	5	5	-3.4953	0.0004735	0.0040566	**
PC(31:1)	value	NL.Out	NO.In	5	5	-3.4942	0.0004756	0.0040566	**
DGDG(36:5)	value	NL.In	NO.Out	5	5	-3.4744	0.0005120	0.0041667	**
DGDG(36:7)	value	NO.In	NO.Out	5	5	-3.4744	0.0005120	0.0041667	**
DGMG(16:1)	value	NL.Out	NO.Out	5	5	-3.4796	0.0005021	0.0041667	**
DGTS(34:5)	value	NO.In	NO.Out	5	5	3.4744	0.0005120	0.0041667	**
LPC(22:5)	value	NL.In	NL.Out	5	5	3.4774	0.0005063	0.0041667	**
PC(34:4)	value	NO.In	NO.Out	5	5	3.4744	0.0005120	0.0041667	**
PC(38:3)	value	NL.In	NO.Out	5	5	-3.4792	0.0005029	0.0041667	**
SQDG(32:1)	value	NL.In	NO.In	5	5	3.4744	0.0005120	0.0041667	**
LPE(18:2)	value	NL.In	NO.Out	5	5	-3.4490	0.0005627	0.0045275	**
PC(37:5)	value	NL.In	NO.Out	5	5	3.4490	0.0005627	0.0045275	**
PI(40:10)	value	NL.In	NO.Out	5	5	3.4404	0.0005809	0.0046468	**
DGMG(20:5)	value	NL.Out	NO.In	5	5	-3.4292	0.0006054	0.0047887	**
PC(36:4(OH))	value	NO.In	NO.Out	5	5	-3.4300	0.0006036	0.0047887	**
DGTS(34:4)	value	NL.In	NO.Out	5	5	3.4222	0.0006211	0.0048024	**
DGTS(36:8)	value	NL.Out	NO.In	5	5	-3.4209	0.0006240	0.0048024	**
PC(38:8)	value	NL.Out	NO.In	5	5	-3.4209	0.0006240	0.0048024	**
PG(31:1)	value	NL.Out	NO.In	5	5	-3.4213	0.0006231	0.0048024	**
PI-Cer(d18:1/14:1)	value	NL.In	NO.Out	5	5	3.4209	0.0006240	0.0048024	**
PC(38:2)	value	NL.In	NL.Out	5	5	-3.4045	0.0006629	0.0050740	**
PC(38:5)	value	NL.Out	NO.In	5	5	3.3955	0.0006850	0.0052152	**
PI(34:5)	value	NL.Out	NO.In	5	5	3.3828	0.0007175	0.0054334	**
DGTS(36:6)	value	NO.In	NO.Out	5	5	3.3688	0.0007551	0.0056236	**
DGTS(38:5)	value	NL.In	NO.Out	5	5	-3.3700	0.0007516	0.0056236	**
DGTS(40:10)	value	NL.Out	NO.In	5	5	-3.3675	0.0007586	0.0056236	**
PC(32:1)	value	NO.In	NO.Out	5	5	3.3675	0.0007586	0.0056236	**
PE(30:1)	value	NL.In	NL.Out	5	5	3.3456	0.0008210	0.0060231	**
PE(36:4)	value	NO.In	NO.Out	5	5	3.3458	0.0008205	0.0060231	**
PG(32:2)	value	NL.Out	NO.In	5	5	-3.3392	0.0008401	0.0061002	**
PG(32:2)	value	NO.In	NO.Out	5	5	3.3392	0.0008401	0.0061002	**
PI(34:5)	value	NL.In	NO.In	5	5	3.3291	0.0008713	0.0062947	**
DGMG(16:0)	value	NL.Out	NO.In	5	5	-3.3241	0.0008872	0.0063448	**
PE(32:1)	value	NL.Out	NO.Out	5	5	-3.3241	0.0008872	0.0063448	**
DGTS(36:6)	value	NL.In	NO.Out	5	5	3.3153	0.0009155	0.0064783	**
DGTS(38:6)	value	NL.In	NO.Out	5	5	-3.3140	0.0009196	0.0064783	**
PG(32:1)	value	NL.Out	NO.In	5	5	-3.3153	0.0009155	0.0064783	**
PC(38:2)	value	NL.In	NO.Out	5	5	-3.2972	0.0009764	0.0068106	**
SQDG(28:0)	value	NL.Out	NO.In	5	5	-3.2972	0.0009764	0.0068106	**
PC(38:5)	value	NO.In	NO.Out	5	5	-3.2885	0.0010070	0.0069901	**
PC(30:3)	value	NL.In	NL.Out	5	5	3.2798	0.0010388	0.0071575	**
PG(33:1)	value	NO.In	NO.Out	5	5	3.2791	0.0010413	0.0071575	**
DGMG(16:0)	value	NL.Out	NO.Out	5	5	-3.2704	0.0010738	0.0073455	**
LPG(16:0)	value	NL.Out	NO.In	5	5	3.2643	0.0010975	0.0074712	**
PC(38:6)	value	NL.In	NL.Out	5	5	-3.2606	0.0011118	0.0074968	**
PI(34:1)	value	NL.In	NL.Out	5	5	3.2606	0.0011118	0.0074968	**
PE(38:9)	value	NL.Out	NO.In	5	5	-3.2585	0.0011202	0.0075176	**

LPE(20:3)	value	NO.In	NO.Out	5	5	3.2351	0.0012161	0.0080845	**
SQDG(32:2)	value	NL.Out	NO.In	5	5	3.2351	0.0012161	0.0080845	**
PG(31:1)	value	NO.In	NO.Out	5	5	3.2328	0.0012260	0.0081123	**
PE(32:4)	value	NL.Out	NO.In	5	5	-3.2210	0.0012776	0.0084146	**
PC(36:4(OH))	value	NL.Out	NO.In	5	5	3.2156	0.0013017	0.0085333	**
PC(32:1)	value	NL.Out	NO.In	5	5	-3.2071	0.0013406	0.0086683	**
PC(34:2)	value	NL.In	NL.Out	5	5	-3.2071	0.0013406	0.0086683	**
PI(34:2)	value	NL.In	NO.Out	5	5	3.2071	0.0013406	0.0086683	**
PE(38:7)	value	NO.In	NO.Out	5	5	-3.1876	0.0014346	0.0092333	**
DGDG(30:1)	value	NL.In	NO.In	5	5	-3.1561	0.0015992	0.0100570	*
DGTS(34:3)	value	NL.Out	NO.Out	5	5	3.1549	0.0016057	0.0100570	*
DGTS(36:8)	value	NO.In	NO.Out	5	5	3.1537	0.0016122	0.0100570	*
DGTS(38:6)	value	NL.In	NL.Out	5	5	-3.1537	0.0016122	0.0100570	*
MGTS(20:4)	value	NL.Out	NO.In	5	5	3.1537	0.0016122	0.0100570	*
PC(34:2)	value	NL.In	NO.Out	5	5	-3.1537	0.0016122	0.0100570	*
PG(32:1)	value	NL.In	NO.In	5	5	-3.1549	0.0016057	0.0100570	*
DGDG(32:3)	value	NL.In	NL.Out	5	5	-3.1400	0.0016897	0.0104937	*
PE(38:7)	value	NL.Out	NO.In	5	5	3.1340	0.0017242	0.0106615	*
PG(35:5)	value	NL.Out	NO.In	5	5	3.1293	0.0017522	0.0107406	*
SQDG(34:1)	value	NL.In	NO.In	5	5	3.1305	0.0017452	0.0107406	*
LPE(14:0)	value	NL.In	NL.Out	5	5	3.1258	0.0017734	0.0108240	*
MGTS(14:1)	value	NL.In	NL.Out	5	5	3.1220	0.0017963	0.0109163	*
MGDG(34:1)	value	NL.Out	NO.In	5	5	3.1126	0.0018544	0.0111736	*
MGDG(34:1)	value	NO.In	NO.Out	5	5	-3.1126	0.0018544	0.0111736	*
DGTS(38:7)	value	NO.In	NO.Out	5	5	-3.1014	0.0019261	0.0113683	*
DGTS(40:10)	value	NO.In	NO.Out	5	5	3.1002	0.0019337	0.0113683	*
DGTS(40:9)	value	NL.In	NO.Out	5	5	3.1002	0.0019337	0.0113683	*
LPC(17:2)	value	NL.Out	NO.In	5	5	-3.1001	0.0019349	0.0113683	*
PE(32:4)	value	NL.In	NL.Out	5	5	3.1038	0.0019103	0.0113683	*
PI(34:1)	value	NL.In	NO.In	5	5	3.1002	0.0019337	0.0113683	*
DGDG(32:5)	value	NL.In	NO.Out	5	5	3.0828	0.0020507	0.0119966	*
PC(35:1)	value	NO.In	NO.Out	5	5	-3.0816	0.0020587	0.0119966	*
LPC(20:5)	value	NL.Out	NO.In	5	5	-3.0747	0.0021074	0.0120327	*
PC(36:7)	value	NO.In	NO.Out	5	5	3.0758	0.0020993	0.0120327	*
PE(30:1)	value	NL.Out	NO.In	5	5	-3.0758	0.0020992	0.0120327	*
PE(36:4)	value	NL.Out	NO.In	5	5	-3.0781	0.0020830	0.0120327	*
LPG(16:0)	value	NL.In	NO.In	5	5	3.0770	0.0020911	0.0120327	*
DGTS(32:5)	value	NO.In	NO.Out	5	5	3.0664	0.0021663	0.0122697	*
DGTS(38:10)	value	NL.Out	NO.In	5	5	-3.0676	0.0021578	0.0122697	*
PC(28:1)	value	NL.Out	NO.In	5	5	-3.0644	0.0021811	0.0123046	*
PG(36:6)	value	NL.In	NO.Out	5	5	3.0555	0.0022468	0.0125752	*
PG(36:6)	value	NO.In	NO.Out	5	5	3.0555	0.0022468	0.0125752	*
DGDG(32:2)	value	NL.In	NL.Out	5	5	-3.0468	0.0023131	0.0125974	*
DGTS(34:4)	value	NL.Out	NO.Out	5	5	3.0479	0.0023043	0.0125974	*
LPC(17:1)	value	NL.In	NL.Out	5	5	3.0488	0.0022972	0.0125974	*
LPE(20:4)	value	NL.Out	NO.In	5	5	-3.0468	0.0023131	0.0125974	*
MGTS(20:5)	value	NL.Out	NO.In	5	5	-3.0468	0.0023131	0.0125974	*
PG(36:5)	value	NL.In	NO.Out	5	5	-3.0468	0.0023131	0.0125974	*
PI(34:2)	value	NL.In	NL.Out	5	5	3.0468	0.0023131	0.0125974	*
PG(33:1)	value	NL.In	NO.In	5	5	-3.0372	0.0023876	0.0129537	*
DGDG(32:3)	value	NL.In	NO.Out	5	5	-3.0326	0.0024244	0.0131029	*

LPC(22:5)	value	NL.Out	NO.Out	5	5	-3.0238	0.0024962	0.0134394	*
PG(31:0)	value	NL.Out	NO.In	5	5	-3.0161	0.0025606	0.0137340	*
PC(34:7)	value	NO.In	NO.Out	5	5	3.0134	0.0025833	0.0138035	*
DGDG(32:2)	value	NL.In	NO.Out	5	5	-2.9933	0.0027595	0.0141577	*
DGDG(35:5)	value	NO.In	NO.Out	5	5	-2.9933	0.0027595	0.0141577	*
DGTS(34:5)	value	NL.In	NO.Out	5	5	2.9933	0.0027595	0.0141577	*
MGTS(16:3)	value	NL.In	NO.Out	5	5	2.9933	0.0027595	0.0141577	*
MGTS(18:3)	value	NO.In	NO.Out	5	5	-2.9945	0.0027494	0.0141577	*
MGTS(20:4)	value	NL.In	NO.In	5	5	2.9933	0.0027595	0.0141577	*
PC(33:2)	value	NL.In	NO.Out	5	5	2.9945	0.0027494	0.0141577	*
PC(35:4)	value	NL.In	NO.In	5	5	-2.9967	0.0027291	0.0141577	*
PC(38:7)	value	NL.Out	NO.In	5	5	-2.9945	0.0027494	0.0141577	*
PE(40:7)	value	NL.In	NO.Out	5	5	2.9945	0.0027494	0.0141577	*
SQDG(32:1)	value	NL.In	NO.Out	5	5	2.9933	0.0027595	0.0141577	*
PC(37:6)	value	NL.In	NO.Out	5	5	2.9744	0.0029353	0.0150048	*
PC(32:4)	value	NL.Out	NO.In	5	5	-2.9722	0.0029568	0.0150606	*
DGDG(38:6)	value	NL.In	NO.In	5	5	2.9700	0.0029785	0.0151165	*
LPC(18:3)	value	NL.Out	NO.Out	5	5	-2.9688	0.0029893	0.0151175	*
PI(40:10)	value	NL.Out	NO.Out	5	5	2.9566	0.0031106	0.0156747	*
DGDG(30:1)	value	NL.In	NL.Out	5	5	-2.9421	0.0032601	0.0161987	*
DGTS(33:2)	value	NL.In	NO.In	5	5	2.9432	0.0032484	0.0161987	*
DGTS(38:7)	value	NL.Out	NO.In	5	5	2.9410	0.0032718	0.0161987	*
MGTS(16:2)	value	NL.In	NO.Out	5	5	2.9410	0.0032718	0.0161987	*
PG(30:1)	value	NO.In	NO.Out	5	5	2.9410	0.0032718	0.0161987	*
MGTS(14:0)	value	NL.In	NO.Out	5	5	2.9399	0.0032835	0.0161999	*
PE(38:9)	value	NL.Out	NO.Out	5	5	-2.9353	0.0033322	0.0163835	*
PC(37:4)	value	NL.In	NO.In	5	5	2.9219	0.0034785	0.0170434	*
Cer(d32:1)	value	NL.In	NO.In	5	5	2.9142	0.0035655	0.0173497	*
LPE(20:3)	value	NL.Out	NO.In	5	5	-2.9142	0.0035655	0.0173497	*
PC(31:2)	value	NL.In	NO.Out	5	5	2.9003	0.0037278	0.0180772	*
PC(28:1)	value	NO.In	NO.Out	5	5	2.8941	0.0038019	0.0183735	*
DGTS(34:6)	value	NO.In	NO.Out	5	5	2.8930	0.0038164	0.0183809	*
DGTS(32:1)	value	NL.In	NO.In	5	5	2.8864	0.0038965	0.0183915	*
DGTS(38:5)	value	NL.In	NL.Out	5	5	-2.8886	0.0038697	0.0183915	*
LPE(20:4)	value	NL.In	NO.In	5	5	-2.8864	0.0038965	0.0183915	*
PC(32:5)	value	NO.In	NO.Out	5	5	2.8878	0.0038798	0.0183915	*
PC(38:6)	value	NL.In	NO.Out	5	5	-2.8864	0.0038965	0.0183915	*
PG(36:5)	value	NO.In	NO.Out	5	5	-2.8864	0.0038965	0.0183915	*
DGMG(16:1)	value	NL.In	NL.Out	5	5	2.8640	0.0041832	0.0196792	*
LPC(17:1)	value	NL.Out	NO.Out	5	5	-2.8600	0.0042367	0.0197358	*
LPE(16:2)	value	NL.In	NO.In	5	5	-2.8611	0.0042221	0.0197358	*
MGDG(34:2)	value	NL.In	NL.Out	5	5	-2.8568	0.0042789	0.0197358	*
MGDG(34:2)	value	NL.In	NO.Out	5	5	-2.8568	0.0042789	0.0197358	*
MGDG(34:2)	value	NL.Out	NO.In	5	5	2.8568	0.0042789	0.0197358	*
MGDG(34:2)	value	NO.In	NO.Out	5	5	-2.8568	0.0042789	0.0197358	*
DGTS(30:3)	value	NO.In	NO.Out	5	5	2.8491	0.0043846	0.0200276	*
LPC(17:2)	value	NL.Out	NO.Out	5	5	-2.8509	0.0043590	0.0200276	*
PC(31:1)	value	NO.In	NO.Out	5	5	2.8491	0.0043846	0.0200276	*
DGDG(36:5)	value	NL.Out	NO.Out	5	5	-2.8330	0.0046118	0.0205355	*
LPC(18:2)	value	NL.Out	NO.Out	5	5	-2.8340	0.0045964	0.0205355	*
PC(34:4)	value	NL.Out	NO.In	5	5	-2.8330	0.0046118	0.0205355	*

PC(36:3)	value	NL.In	NO.Out	5	5	-2.8330	0.0046118	0.0205355	*
PC(38:1)	value	NO.In	NO.Out	5	5	-2.8394	0.0045201	0.0205355	*
PE(32:2)	value	NL.In	NL.Out	5	5	2.8340	0.0045964	0.0205355	*
PI-Cer(d18:1/14:1)	value	NO.In	NO.Out	5	5	2.8330	0.0046118	0.0205355	*
SQDG(34:2)	value	NL.In	NO.In	5	5	2.8372	0.0045506	0.0205355	*
LPE(20:2)	value	NL.In	NO.Out	5	5	2.8278	0.0046874	0.0207417	*
LPE(20:2)	value	NL.Out	NO.Out	5	5	2.8278	0.0046874	0.0207417	*
DGTS(38:10)	value	NL.In	NO.In	5	5	-2.8254	0.0047218	0.0208287	*
DGTS(33:1)	value	NL.Out	NO.In	5	5	2.8158	0.0048658	0.0213974	*
DGDG(30:0)	value	NO.In	NO.Out	5	5	-2.8084	0.0049795	0.0216999	*
DGTS(32:3)	value	NL.Out	NO.Out	5	5	2.8084	0.0049795	0.0216999	*
LPE(18:2)	value	NO.In	NO.Out	5	5	-2.8073	0.0049959	0.0216999	*
PC(37:5)	value	NL.In	NO.In	5	5	2.8073	0.0049959	0.0216999	*
PE(32:3)	value	NO.In	NO.Out	5	5	2.8030	0.0050631	0.0219247	*
MGTS(14:1)	value	NL.In	NO.In	5	5	2.7962	0.0051703	0.0223205	*
DGTS(37:5)	value	NL.In	NO.In	5	5	2.7890	0.0052876	0.0227576	*
PC(35:1)	value	NL.In	NO.Out	5	5	-2.7869	0.0053221	0.0228368	*
DGDG(32:1)	value	NO.In	NO.Out	5	5	-2.7795	0.0054440	0.0229425	*
DGTS(28:0)	value	NO.In	NO.Out	5	5	2.7816	0.0054090	0.0229425	*
MGDG(40:10)	value	NL.Out	NO.Out	5	5	-2.7795	0.0054440	0.0229425	*
PC(28:1)	value	NL.In	NL.Out	5	5	2.7807	0.0054250	0.0229425	*
PC(36:3)	value	NL.In	NL.Out	5	5	-2.7795	0.0054440	0.0229425	*
SQDG(32:3)	value	NO.In	NO.Out	5	5	-2.7833	0.0053809	0.0229425	*
PC(37:2)	value	NL.Out	NO.Out	5	5	-2.7632	0.0057238	0.0240501	*
DGDG(34:2)	value	NL.Out	NO.In	5	5	2.7538	0.0058903	0.0244594	*
PC(35:3)	value	NO.In	NO.Out	5	5	-2.7538	0.0058903	0.0244594	*
PC(36:6)	value	NL.Out	NO.In	5	5	-2.7538	0.0058903	0.0244594	*
PG(34:5)	value	NL.In	NO.Out	5	5	2.7538	0.0058903	0.0244594	*
PE(30:0)	value	NL.In	NO.Out	5	5	-2.7502	0.0059556	0.0246582	*
DGDG(34:5)	value	NO.In	NO.Out	5	5	2.7261	0.0064094	0.0250712	*
DGDG(36:6)	value	NO.In	NO.Out	5	5	2.7261	0.0064094	0.0250712	*
DGDG(40:10)	value	NL.In	NL.Out	5	5	2.7261	0.0064094	0.0250712	*
DGTS(32:2)	value	NL.Out	NO.Out	5	5	2.7261	0.0064094	0.0250712	*
LPC(14:0)	value	NL.In	NL.Out	5	5	2.7353	0.0062317	0.0250712	*
LPC(17:0)	value	NL.In	NL.Out	5	5	2.7372	0.0061970	0.0250712	*
LPC(17:0)	value	NO.In	NO.Out	5	5	2.7372	0.0061970	0.0250712	*
LPC(18:1)	value	NO.In	NO.Out	5	5	-2.7261	0.0064094	0.0250712	*
LPC(20:4)	value	NL.In	NO.Out	5	5	-2.7271	0.0063896	0.0250712	*
MGMG(16:0)	value	NL.In	NL.Out	5	5	-2.7312	0.0063104	0.0250712	*
MGTS(18:2)	value	NL.Out	NO.In	5	5	2.7261	0.0064094	0.0250712	*
PC(34:3)	value	NL.In	NL.Out	5	5	-2.7261	0.0064094	0.0250712	*
PC(38:8)	value	NL.In	NL.Out	5	5	2.7261	0.0064094	0.0250712	*
PC(38:9)	value	NL.Out	NO.In	5	5	-2.7261	0.0064094	0.0250712	*
PC(40:5)	value	NL.In	NL.Out	5	5	-2.7384	0.0061730	0.0250712	*
PE(34:3)	value	NL.Out	NO.In	5	5	-2.7353	0.0062317	0.0250712	*
PE(34:5)	value	NL.In	NL.Out	5	5	2.7333	0.0062709	0.0250712	*
PI(33:1)	value	NL.Out	NO.Out	5	5	-2.7302	0.0063301	0.0250712	*
SQDG(31:1)	value	NL.In	NO.Out	5	5	2.7261	0.0064094	0.0250712	*
SQDG(32:3)	value	NL.Out	NO.In	5	5	2.7293	0.0063477	0.0250712	*
PC(38:3)	value	NL.In	NL.Out	5	5	-2.7240	0.0064491	0.0251568	*

DGDG(34:1)	value	NL.In	NO.Out	5	5	-2.6746	0.0074813	0.0251709	*
DGDG(34:1)	value	NL.Out	NO.In	5	5	2.6746	0.0074813	0.0251709	*
DGDG(34:3)	value	NL.In	NL.Out	5	5	-2.6777	0.0074141	0.0251709	*
DGDG(34:3)	value	NO.In	NO.Out	5	5	-2.6777	0.0074141	0.0251709	*
DGTS(28:1)	value	NL.In	NO.In	5	5	2.6919	0.0071044	0.0251709	*
DGTS(28:1)	value	NL.Out	NO.Out	5	5	2.6919	0.0071044	0.0251709	*
DGTS(30:1)	value	NL.In	NO.In	5	5	2.6726	0.0075263	0.0251709	*
DGTS(30:1)	value	NL.Out	NO.Out	5	5	2.6726	0.0075263	0.0251709	*
DGTS(30:2)	value	NL.In	NO.In	5	5	2.6807	0.0073471	0.0251709	*
DGTS(30:2)	value	NL.Out	NO.Out	5	5	2.6807	0.0073471	0.0251709	*
DGTS(32:4)	value	NL.In	NO.In	5	5	2.6726	0.0075263	0.0251709	*
DGTS(32:4)	value	NL.Out	NO.Out	5	5	2.6726	0.0075263	0.0251709	*
DGTS(34:1)	value	NL.In	NO.Out	5	5	-2.6736	0.0075038	0.0251709	*
DGTS(34:1)	value	NL.Out	NO.In	5	5	2.6736	0.0075038	0.0251709	*
DGTS(36:2)	value	NL.In	NL.Out	5	5	2.6732	0.0075141	0.0251709	*
DGTS(36:2)	value	NO.In	NO.Out	5	5	-2.6732	0.0075141	0.0251709	*
Cer(d32:2)	value	NL.In	NO.Out	5	5	-2.6736	0.0075038	0.0251709	*
Cer(d32:2)	value	NL.Out	NO.In	5	5	-2.6736	0.0075038	0.0251709	*
LPC(16:1)	value	NL.In	NL.Out	5	5	2.6726	0.0075263	0.0251709	*
LPC(16:1)	value	NO.In	NO.Out	5	5	2.6726	0.0075263	0.0251709	*
LPC(16:3)	value	NL.In	NL.Out	5	5	2.6721	0.0075371	0.0251709	*
LPC(18:4)	value	NL.In	NL.Out	5	5	2.6726	0.0075263	0.0251709	*
LPC(18:4)	value	NO.In	NO.Out	5	5	2.6726	0.0075263	0.0251709	*
LPC(20:3)	value	NL.In	NO.Out	5	5	2.6726	0.0075263	0.0251709	*
LPC(20:3)	value	NL.Out	NO.In	5	5	-2.6726	0.0075263	0.0251709	*
LPC(22:6)	value	NL.In	NO.Out	5	5	2.6837	0.0072805	0.0251709	*
LPC(22:6)	value	NL.Out	NO.In	5	5	-2.6837	0.0072805	0.0251709	*
LPE(18:1)	value	NL.In	NO.Out	5	5	-2.6726	0.0075263	0.0251709	*
MGDG(32:1)	value	NL.In	NL.Out	5	5	-2.7014	0.0069054	0.0251709	*
MGDG(32:5)	value	NL.In	NO.In	5	5	-2.6929	0.0070826	0.0251709	*
MGDG(32:5)	value	NL.Out	NO.Out	5	5	-2.6929	0.0070826	0.0251709	*
MGDG(38:7)	value	NL.In	NL.Out	5	5	-2.6929	0.0070826	0.0251709	*
MGDG(38:7)	value	NO.In	NO.Out	5	5	-2.6929	0.0070826	0.0251709	*
MGDG(40:9)	value	NL.In	NO.In	5	5	-2.6736	0.0075038	0.0251709	*
MGDG(40:9)	value	NL.Out	NO.Out	5	5	-2.6736	0.0075038	0.0251709	*
MGTS(16:1)	value	NL.In	NO.In	5	5	2.7004	0.0069266	0.0251709	*
MGTS(18:1)	value	NL.In	NO.Out	5	5	-2.6726	0.0075263	0.0251709	*
MGTS(18:1)	value	NL.Out	NO.In	5	5	2.6726	0.0075263	0.0251709	*
MGTS(18:4)	value	NL.In	NO.In	5	5	2.6726	0.0075263	0.0251709	*
PC(34:5)	value	NL.In	NO.Out	5	5	2.6726	0.0075263	0.0251709	*
PC(34:5)	value	NL.Out	NO.In	5	5	-2.6726	0.0075263	0.0251709	*
PC(35:2)	value	NL.Out	NO.In	5	5	2.7004	0.0069266	0.0251709	*
PC(35:3)	value	NL.In	NO.Out	5	5	-2.7004	0.0069266	0.0251709	*
PC(36:2)	value	NL.In	NO.Out	5	5	-2.6726	0.0075263	0.0251709	*
PC(36:2)	value	NL.Out	NO.In	5	5	2.6726	0.0075263	0.0251709	*
PC(36:8)	value	NL.In	NL.Out	5	5	2.6777	0.0074141	0.0251709	*
PC(36:8)	value	NO.In	NO.Out	5	5	2.6777	0.0074141	0.0251709	*
PC(40:8)	value	NL.In	NL.Out	5	5	-2.6726	0.0075263	0.0251709	*
PC(40:8)	value	NO.In	NO.Out	5	5	-2.6726	0.0075263	0.0251709	*
PE(34:2)	value	NL.In	NL.Out	5	5	2.6736	0.0075038	0.0251709	*
PE(34:2)	value	NO.In	NO.Out	5	5	2.6736	0.0075038	0.0251709	*

PE(36:2)	value	NL.In	NO.In	5	5	-2.7179	0.0065688	0.0251709	*
PE(36:2)	value	NL.Out	NO.Out	5	5	-2.7179	0.0065688	0.0251709	*
PE(38:5)	value	NL.In	NO.Out	5	5	-2.6777	0.0074141	0.0251709	*
PE(38:5)	value	NL.Out	NO.In	5	5	2.6777	0.0074141	0.0251709	*
PE(38:6)	value	NL.In	NO.Out	5	5	-2.6726	0.0075263	0.0251709	*
PE(38:6)	value	NL.Out	NO.In	5	5	2.6726	0.0075263	0.0251709	*
PI(32:1)	value	NL.In	NL.Out	5	5	2.6726	0.0075263	0.0251709	*
PI-Cer(d18:1/14:0)	value	NL.In	NO.In	5	5	2.6929	0.0070826	0.0251709	*
PI-Cer(d18:1/14:0)	value	NL.Out	NO.Out	5	5	2.6929	0.0070826	0.0251709	*
SQDG(28:0)	value	NL.In	NO.In	5	5	-2.7075	0.0067793	0.0251709	*
PC(38:3)	value	NO.In	NO.Out	5	5	-2.6701	0.0075832	0.0252653	*
PE(36:7)	value	NL.Out	NO.Out	5	5	-2.6626	0.0077550	0.0257771	*
LPE(16:0)	value	NL.In	NO.Out	5	5	-2.6569	0.0078864	0.0260005	*
LPE(16:0)	value	NL.Out	NO.In	5	5	-2.6569	0.0078864	0.0260005	*
PC(37:3)	value	NL.In	NO.Out	5	5	-2.6559	0.0079099	0.0260005	*
PG(36:6)	value	NL.In	NL.Out	5	5	2.6557	0.0079140	0.0260005	*
PG(36:6)	value	NL.Out	NO.In	5	5	-2.6557	0.0079140	0.0260005	*
PC(37:7)	value	NO.In	NO.Out	5	5	2.6549	0.0079336	0.0260045	*
PC(34:6)	value	NL.In	NO.Out	5	5	2.6519	0.0080046	0.0261165	*
PC(34:6)	value	NL.Out	NO.In	5	5	-2.6519	0.0080046	0.0261165	*
MGDG(32:1)	value	NO.In	NO.Out	5	5	-2.6479	0.0080999	0.0263233	*
PC(35:2)	value	NL.In	NO.Out	5	5	-2.6469	0.0081238	0.0263233	*
PC(35:3)	value	NL.Out	NO.In	5	5	2.6469	0.0081238	0.0263233	*
PE(32:3)	value	NL.In	NL.Out	5	5	2.6381	0.0083370	0.0269524	*
DGDG(40:10)	value	NO.In	NO.Out	5	5	2.6192	0.0088147	0.0271338	*
DGTS(32:2)	value	NL.In	NO.In	5	5	2.6192	0.0088147	0.0271338	*
LPC(14:0)	value	NO.In	NO.Out	5	5	2.6281	0.0085872	0.0271338	*
LPC(16:2)	value	NL.In	NL.Out	5	5	2.6211	0.0087638	0.0271338	*
LPC(16:2)	value	NO.In	NO.Out	5	5	2.6211	0.0087638	0.0271338	*
MGDG(36:5)	value	NL.In	NL.Out	5	5	-2.6211	0.0087638	0.0271338	*
MGDG(36:5)	value	NO.In	NO.Out	5	5	-2.6211	0.0087638	0.0271338	*
MGMG(16:0)	value	NO.In	NO.Out	5	5	-2.6241	0.0086879	0.0271338	*
MGTS(16:0)	value	NL.In	NL.Out	5	5	2.6192	0.0088147	0.0271338	*
MGTS(16:0)	value	NO.In	NO.Out	5	5	-2.6192	0.0088147	0.0271338	*
PC(30:3)	value	NL.In	NO.Out	5	5	2.6293	0.0085570	0.0271338	*
PC(34:3)	value	NO.In	NO.Out	5	5	2.6192	0.0088147	0.0271338	*
PC(35:4)	value	NL.Out	NO.In	5	5	-2.6221	0.0087385	0.0271338	*
PC(36:5)	value	NO.In	NO.Out	5	5	2.6192	0.0088147	0.0271338	*
PC(37:6)	value	NO.In	NO.Out	5	5	2.6261	0.0086375	0.0271338	*
PC(38:10)	value	NO.In	NO.Out	5	5	2.6301	0.0085370	0.0271338	*
PC(38:8)	value	NO.In	NO.Out	5	5	2.6192	0.0088147	0.0271338	*
PC(38:9)	value	NL.In	NO.Out	5	5	2.6192	0.0088147	0.0271338	*
PC(40:5)	value	NO.In	NO.Out	5	5	-2.6311	0.0085120	0.0271338	*
PE(32:1)	value	NL.Out	NO.In	5	5	-2.6271	0.0086123	0.0271338	*
PE(34:5)	value	NO.In	NO.Out	5	5	2.6261	0.0086375	0.0271338	*
SQDG(31:1)	value	NL.Out	NO.In	5	5	2.6192	0.0088147	0.0271338	*
PC(28:1)	value	NL.In	NO.Out	5	5	2.6104	0.0090434	0.0277775	*
PC(37:7)	value	NL.In	NL.Out	5	5	2.6013	0.0092885	0.0284686	*
MGDG(34:1)	value	NL.In	NL.Out	5	5	-2.5986	0.0093603	0.0285652	*
MGDG(34:1)	value	NL.In	NO.Out	5	5	-2.5986	0.0093603	0.0285652	*

DGDG(34:2)	value	NL.In	NO.Out	5	5	-2.5934	0.0095030	0.0286912	*
MGTS(16:1)	value	NL.Out	NO.Out	5	5	2.5934	0.0095030	0.0286912	*
MGTS(18:5)	value	NL.Out	NO.In	5	5	2.5944	0.0094760	0.0286912	*
PC(35:3)	value	NL.In	NL.Out	5	5	-2.5934	0.0095030	0.0286912	*
PG(36:2)	value	NL.Out	NO.In	5	5	-2.5944	0.0094760	0.0286912	*
MGDG(30:1)	value	NL.In	NL.Out	5	5	-2.5862	0.0097039	0.0291734	*
MGDG(30:1)	value	NO.In	NO.Out	5	5	-2.5862	0.0097039	0.0291734	*
PE(36:8)	value	NL.In	NL.Out	5	5	2.5831	0.0097929	0.0293788	*
PE(34:3)	value	NL.In	NO.Out	5	5	2.5744	0.0100404	0.0300577	*
DGDG(32:1)	value	NL.In	NL.Out	5	5	-2.5657	0.0102965	0.0301862	*
DGTS(28:0)	value	NL.In	NL.Out	5	5	2.5676	0.0102394	0.0301862	*
LPC(22:5)	value	NL.Out	NO.In	5	5	-2.5702	0.0101629	0.0301862	*
MGDG(40:10)	value	NL.In	NO.In	5	5	-2.5657	0.0102965	0.0301862	*
PC(32:3)	value	NL.In	NO.Out	5	5	2.5657	0.0102965	0.0301862	*
PC(32:3)	value	NL.Out	NO.In	5	5	-2.5657	0.0102965	0.0301862	*
PC(36:3)	value	NO.In	NO.Out	5	5	-2.5657	0.0102965	0.0301862	*
PG(34:1)	value	NL.In	NL.Out	5	5	-2.5657	0.0102965	0.0301862	*
SQDG(32:2)	value	NL.In	NO.In	5	5	2.5667	0.0102679	0.0301862	*
SQDG(34:1)	value	NL.Out	NO.In	5	5	2.5686	0.0102108	0.0301862	*
LPC(16:3)	value	NO.In	NO.Out	5	5	2.5642	0.0103423	0.0302577	*
PC(32:5)	value	NL.In	NL.Out	5	5	2.5609	0.0104415	0.0304850	*
PC(37:3)	value	NL.Out	NO.In	5	5	2.5486	0.0108162	0.0315139	*
PE(36:6)	value	NL.Out	NO.Out	5	5	-2.5447	0.0109363	0.0317983	*
DGTS(32:3)	value	NL.In	NO.In	5	5	2.5409	0.0110570	0.0320834	*
PG(34:5)	value	NL.Out	NO.In	5	5	-2.5399	0.0110872	0.0321054	*
DGTS(30:3)	value	NL.In	NL.Out	5	5	2.5265	0.0115191	0.0332201	*
PC(31:1)	value	NL.In	NL.Out	5	5	2.5265	0.0115191	0.0332201	*
DGDG(34:5)	value	NL.In	NO.In	5	5	-2.5123	0.0119962	0.0332420	*
LPC(16:0)	value	NL.Out	NO.Out	5	5	-2.5123	0.0119962	0.0332420	*
LPC(18:1)	value	NL.In	NL.Out	5	5	-2.5123	0.0119962	0.0332420	*
LPC(18:2)	value	NL.In	NO.In	5	5	-2.5132	0.0119641	0.0332420	*
LPE(16:1)	value	NL.In	NL.Out	5	5	2.5123	0.0119962	0.0332420	*
MGDG(34:5)	value	NL.In	NL.Out	5	5	-2.5132	0.0119641	0.0332420	*
MGDG(34:5)	value	NO.In	NO.Out	5	5	-2.5132	0.0119641	0.0332420	*
MGTS(15:0)	value	NL.In	NO.In	5	5	2.5160	0.0118681	0.0332420	*
MGTS(15:0)	value	NL.Out	NO.Out	5	5	-2.5160	0.0118681	0.0332420	*
PC(34:1)	value	NL.In	NO.Out	5	5	-2.5123	0.0119962	0.0332420	*
PC(34:1)	value	NL.Out	NO.In	5	5	2.5123	0.0119962	0.0332420	*
PC(36:3)	value	NL.Out	NO.In	5	5	2.5123	0.0119962	0.0332420	*
PC(36:4)	value	NL.Out	NO.In	5	5	2.5123	0.0119962	0.0332420	*
PC(36:5)	value	NL.In	NL.Out	5	5	2.5123	0.0119962	0.0332420	*
PC(38:1)	value	NL.In	NL.Out	5	5	-2.5179	0.0118043	0.0332420	*
PC(38:10)	value	NL.In	NL.Out	5	5	2.5227	0.0116455	0.0332420	*
PE(32:2)	value	NO.In	NO.Out	5	5	2.5132	0.0119641	0.0332420	*
PE(40:7)	value	NL.Out	NO.Out	5	5	2.5132	0.0119641	0.0332420	*
PE(40:7)	value	NO.In	NO.Out	5	5	2.5132	0.0119641	0.0332420	*
SQDG(34:2)	value	NL.Out	NO.Out	5	5	2.5160	0.0118681	0.0332420	*
DGTS(36:7)	value	NL.Out	NO.Out	5	5	2.4874	0.0128680	0.0355188	*
DGTS(38:9)	value	NL.In	NO.Out	5	5	2.4874	0.0128680	0.0355188	*
PC(36:6)	value	NL.In	NO.Out	5	5	2.4865	0.0129019	0.0355431	*
PI(30:1)	value	NL.In	NL.Out	5	5	2.4775	0.0132306	0.0363072	*

PI(30:1)	value	NO.In	NO.Out	5	5	2.4775	0.0132306	0.0363072	*
DGTS(32:1)	value	NL.Out	NO.Out	5	5	2.4588	0.0139401	0.0373141	*
DGTS(34:6)	value	NL.In	NL.Out	5	5	2.4644	0.0137255	0.0373141	*
DGTS(36:4)	value	NL.In	NO.Out	5	5	-2.4588	0.0139401	0.0373141	*
DGTS(38:5)	value	NO.In	NO.Out	5	5	-2.4607	0.0138684	0.0373141	*
LPE(18:4)	value	NL.In	NL.Out	5	5	2.4634	0.0137612	0.0373141	*
LPE(20:5)	value	NL.In	NL.Out	5	5	2.4588	0.0139401	0.0373141	*
LPE(20:5)	value	NO.In	NO.Out	5	5	-2.4588	0.0139401	0.0373141	*
PC(31:2)	value	NL.Out	NO.In	5	5	-2.4666	0.0136391	0.0373141	*
PC(38:6)	value	NL.Out	NO.In	5	5	2.4588	0.0139401	0.0373141	*
PE(32:4)	value	NL.Out	NO.Out	5	5	-2.4596	0.0139077	0.0373141	*
PE(40:8)	value	NL.In	NL.Out	5	5	-2.4588	0.0139401	0.0373141	*
PG(36:5(OH))	value	NL.In	NL.Out	5	5	2.4597	0.0139042	0.0373141	*
PG(36:5(OH))	value	NO.In	NO.Out	5	5	2.4597	0.0139042	0.0373141	*
LPE(18:3)	value	NL.In	NO.In	5	5	-2.4385	0.0147482	0.0394028	*
PE(34:6)	value	NO.In	NO.Out	5	5	2.4367	0.0148204	0.0395211	*
SQDG(36:5)	value	NL.In	NO.In	5	5	2.4357	0.0148612	0.0395555	*
DGDG(30:0)	value	NL.In	NL.Out	5	5	-2.4339	0.0149368	0.0396077	*
DGTS(38:9)	value	NL.Out	NO.In	5	5	-2.4339	0.0149368	0.0396077	*
LPE(20:3)	value	NL.In	NO.Out	5	5	2.4330	0.0149746	0.0396338	*
DGMG(20:5)	value	NL.In	NL.Out	5	5	2.4301	0.0150928	0.0398720	*
PE(36:3)	value	NL.In	NO.Out	5	5	-2.4241	0.0153483	0.0400982	*
PE(36:3)	value	NL.Out	NO.In	5	5	-2.4241	0.0153483	0.0400982	*
PI(34:3)	value	NL.In	NL.Out	5	5	2.4246	0.0153266	0.0400982	*
PI(34:3)	value	NO.In	NO.Out	5	5	2.4246	0.0153266	0.0400982	*
PI(36:6)	value	NL.In	NL.Out	5	5	2.4255	0.0152875	0.0400982	*
PI(36:6)	value	NO.In	NO.Out	5	5	2.4255	0.0152875	0.0400982	*
PI(40:10)	value	NL.In	NO.In	5	5	2.4190	0.0155619	0.0405813	*
MGTS(17:2)	value	NL.In	NO.In	5	5	2.4181	0.0156013	0.0406092	*
PC(34:7)	value	NL.In	NL.Out	5	5	2.4162	0.0156852	0.0407529	*
DGDG(36:6)	value	NL.In	NL.Out	5	5	2.4054	0.0161569	0.0412964	*
DGTS(33:2)	value	NL.Out	NO.Out	5	5	2.4081	0.0160372	0.0412964	*
DGTS(38:7)	value	NL.In	NO.Out	5	5	-2.4063	0.0161170	0.0412964	*
DGTS(38:8)	value	NL.Out	NO.In	5	5	-2.4054	0.0161569	0.0412964	*
MGTS(14:0)	value	NL.Out	NO.In	5	5	-2.4054	0.0161569	0.0412964	*
MGTS(16:2)	value	NL.Out	NO.In	5	5	2.4063	0.0161170	0.0412964	*
PC(38:7)	value	NL.In	NO.In	5	5	-2.4063	0.0161170	0.0412964	*
PE(40:10)	value	NL.Out	NO.In	5	5	2.4063	0.0161170	0.0412964	*
SQDG(32:2)	value	NL.Out	NO.Out	5	5	2.4063	0.0161170	0.0412964	*
PC(32:4)	value	NL.In	NO.Out	5	5	2.3831	0.0171671	0.0437994	*
DGDG(38:6)	value	NL.Out	NO.Out	5	5	2.3813	0.0172511	0.0439344	*
LPC(18:3)	value	NL.In	NO.In	5	5	-2.3804	0.0172931	0.0439624	*
LPC(18:5)	value	NL.In	NL.Out	5	5	2.3683	0.0178700	0.0452663	*
LPC(18:5)	value	NO.In	NO.Out	5	5	2.3683	0.0178700	0.0452663	*
DGDG(32:2)	value	NL.Out	NO.In	5	5	2.3519	0.0186778	0.0459962	*
DGDG(35:5)	value	NL.In	NL.Out	5	5	-2.3519	0.0186778	0.0459962	*
DGTS(34:5)	value	NL.Out	NO.In	5	5	-2.3519	0.0186778	0.0459962	*
DGTS(36:4)	value	NL.Out	NO.In	5	5	2.3519	0.0186778	0.0459962	*
LPC(17:2)	value	NL.In	NL.Out	5	5	2.3527	0.0186368	0.0459962	*
LPE(18:4)	value	NO.In	NO.Out	5	5	2.3563	0.0184564	0.0459962	*
MGDG(40:8)	value	NL.In	NL.Out	5	5	-2.3608	0.0182360	0.0459962	*

MGDG(40:8)	value	NO.In	NO.Out	5	5	-2.3608	0.0182360	0.0459962	*
MGTS(16:3)	value	NL.Out	NO.In	5	5	-2.3519	0.0186778	0.0459962	*
MGTS(18:3)	value	NL.In	NL.Out	5	5	2.3528	0.0186335	0.0459962	*
MGTS(18:4)	value	NL.Out	NO.Out	5	5	2.3519	0.0186778	0.0459962	*
MGTS(20:4)	value	NL.Out	NO.Out	5	5	2.3519	0.0186778	0.0459962	*
PC(32:2)	value	NL.In	NO.Out	5	5	2.3519	0.0186778	0.0459962	*
PC(32:2)	value	NL.Out	NO.In	5	5	-2.3519	0.0186778	0.0459962	*
PG(34:1)	value	NO.In	NO.Out	5	5	-2.3519	0.0186778	0.0459962	*
PI(33:1)	value	NO.In	NO.Out	5	5	-2.3554	0.0185006	0.0459962	*
PE(38:9)	value	NL.In	NO.In	5	5	-2.3429	0.0191368	0.0470447	*
DGDG(32:3)	value	NL.Out	NO.In	5	5	2.3348	0.0195514	0.0478974	*
PE(30:3)	value	NL.In	NO.In	5	5	-2.3348	0.0195514	0.0478974	*
DGTS(33:1)	value	NL.In	NO.Out	5	5	2.3331	0.0196439	0.0480411	*
LPE(16:3)	value	NL.In	NL.Out	5	5	2.3279	0.0199197	0.0481410	*
LPE(18:3)	value	NL.Out	NO.Out	5	5	-2.3313	0.0197367	0.0481410	*
PC(30:1)	value	NL.In	NO.Out	5	5	2.3278	0.0199227	0.0481410	*
PC(30:1)	value	NL.Out	NO.In	5	5	-2.3278	0.0199227	0.0481410	*
PE(36:5)	value	NL.In	NO.In	5	5	-2.3287	0.0198762	0.0481410	*
PE(36:6)	value	NL.In	NO.In	5	5	-2.3304	0.0197832	0.0481410	*
SQDG(34:1)	value	NO.In	NO.Out	5	5	-2.3278	0.0199227	0.0481410	*
DGTS(34:2)	value	NO.In	NO.Out	5	5	-2.3260	0.0200160	0.0482840	*
LPE(16:2)	value	NL.Out	NO.Out	5	5	-2.3212	0.0202735	0.0488219	*
MGTS(17:2)	value	NL.Out	NO.Out	5	5	2.3106	0.0208527	0.0500466	ns
PE(36:8)	value	NO.In	NO.Out	5	5	2.3112	0.0208243	0.0500466	ns
MGDG(32:2)	value	NL.In	NL.Out	5	5	-2.3028	0.0212913	0.0509264	ns
MGDG(32:2)	value	NO.In	NO.Out	5	5	-2.3028	0.0212913	0.0509264	ns
DGDG(32:2)	value	NO.In	NO.Out	5	5	-2.2984	0.0215364	0.0510813	ns
DGDG(36:7)	value	NL.Out	NO.In	5	5	2.2984	0.0215364	0.0510813	ns
DGTS(34:4)	value	NL.In	NO.In	5	5	2.2993	0.0214873	0.0510813	ns
DGTS(38:8)	value	NL.In	NO.Out	5	5	-2.2984	0.0215364	0.0510813	ns
LPE(16:1)	value	NO.In	NO.Out	5	5	2.2984	0.0215364	0.0510813	ns
MGTS(16:4)	value	NL.In	NO.Out	5	5	2.2800	0.0226069	0.0535308	ns
PE(36:4)	value	NL.In	NO.Out	5	5	2.2751	0.0228973	0.0541278	ns
PG(35:5)	value	NO.In	NO.Out	5	5	-2.2734	0.0230002	0.0541903	ns
PG(36:2)	value	NL.In	NO.Out	5	5	-2.2734	0.0230002	0.0541903	ns
LPC(20:5)	value	NL.In	NO.Out	5	5	-2.2726	0.0230518	0.0542215	ns
PE(34:6)	value	NL.In	NL.Out	5	5	2.2706	0.0231714	0.0544125	ns
LPC(20:0)	value	NL.In	NO.Out	5	5	-2.2655	0.0234803	0.0549555	ns
LPC(20:0)	value	NL.Out	NO.In	5	5	-2.2655	0.0234803	0.0549555	ns
PG(31:0)	value	NL.In	NO.Out	5	5	2.2621	0.0236933	0.0553625	ns
DGDG(32:5)	value	NL.Out	NO.Out	5	5	2.2518	0.0243364	0.0567715	ns
DGTS(38:7)	value	NL.In	NL.Out	5	5	-2.2458	0.0247143	0.0570279	ns
DGTS(40:10)	value	NL.In	NL.Out	5	5	2.2450	0.0247685	0.0570279	ns
DGTS(40:9)	value	NL.Out	NO.In	5	5	-2.2450	0.0247685	0.0570279	ns
MGDG(36:6)	value	NL.In	NO.In	5	5	-2.2467	0.0246602	0.0570279	ns
MGDG(36:6)	value	NL.Out	NO.Out	5	5	-2.2467	0.0246602	0.0570279	ns
PC(33:3)	value	NL.In	NO.Out	5	5	2.2475	0.0246061	0.0570279	ns
PC(33:3)	value	NL.Out	NO.In	5	5	-2.2475	0.0246061	0.0570279	ns
PI(32:1)	value	NO.In	NO.Out	5	5	-2.2450	0.0247685	0.0570279	ns
DGMG(20:5)	value	NL.Out	NO.Out	5	5	-2.2411	0.0250174	0.0575076	ns
PE(34:4)	value	NL.In	NL.Out	5	5	2.2326	0.0255765	0.0586974	ns

PC(40:11)	value	NO.In	NO.Out	5	5	2.2283	0.0258576	0.0592465	ns
DGDG(32:3)	value	NO.In	NO.Out	5	5	-2.2275	0.0259139	0.0592797	ns
MGTS(14:1)	value	NL.In	NO.Out	5	5	2.2261	0.0260059	0.0593942	ns
MGTS(16:4)	value	NL.Out	NO.In	5	5	-2.2237	0.0261676	0.0596671	ns
SQDG(36:5)	value	NL.Out	NO.Out	5	5	-2.2216	0.0263096	0.0598944	ns
DGDG(38:7)	value	NL.In	NO.In	5	5	2.2199	0.0264230	0.0600561	ns
DGTS(32:5)	value	NL.In	NL.Out	5	5	2.2057	0.0274061	0.0621908	ns
PE(30:0)	value	NL.Out	NO.In	5	5	-2.2002	0.0277946	0.0629714	ns
DGTS(37:5)	value	NL.Out	NO.Out	5	5	-2.1990	0.0278783	0.0630601	ns
DGTS(34:3)	value	NL.In	NO.In	5	5	2.1924	0.0283531	0.0633578	ns
DGTS(36:8)	value	NL.In	NL.Out	5	5	2.1915	0.0284126	0.0633578	ns
DGTS(38:6)	value	NO.In	NO.Out	5	5	-2.1915	0.0284126	0.0633578	ns
MGTS(20:4)	value	NL.In	NO.Out	5	5	2.1915	0.0284126	0.0633578	ns
MGTS(20:5)	value	NL.In	NO.Out	5	5	2.1915	0.0284126	0.0633578	ns
PC(34:2)	value	NL.Out	NO.In	5	5	2.1915	0.0284126	0.0633578	ns
PC(35:4)	value	NL.In	NO.Out	5	5	-2.1940	0.0282341	0.0633578	ns
PC(40:10)	value	NL.In	NO.In	5	5	-2.1924	0.0283531	0.0633578	ns
PC(40:10)	value	NL.Out	NO.Out	5	5	-2.1924	0.0283531	0.0633578	ns
LPC(17:1)	value	NL.Out	NO.In	5	5	-2.1855	0.0288556	0.0642446	ns
PC(40:11)	value	NL.In	NL.Out	5	5	2.1746	0.0296564	0.0659239	ns
PE(30:3)	value	NL.Out	NO.Out	5	5	-2.1738	0.0297183	0.0659579	ns
DGDG(32:5)	value	NO.In	NO.Out	5	5	2.1714	0.0299042	0.0662667	ns
PC(35:1)	value	NL.Out	NO.Out	5	5	-2.1705	0.0299663	0.0663004	ns
PC(36:7)	value	NL.In	NL.Out	5	5	2.1664	0.0302772	0.0668837	ns
MGTS(17:1)	value	NL.In	NO.Out	5	5	2.1462	0.0318591	0.0701595	ns
MGTS(17:1)	value	NL.Out	NO.In	5	5	2.1462	0.0318591	0.0701595	ns
DGTS(36:5)	value	NL.Out	NO.In	5	5	-2.1381	0.0325094	0.0710392	ns
LPE(18:1)	value	NL.Out	NO.In	5	5	2.1381	0.0325094	0.0710392	ns
PC(34:2)	value	NO.In	NO.Out	5	5	-2.1381	0.0325094	0.0710392	ns
PE(40:8)	value	NO.In	NO.Out	5	5	2.1381	0.0325094	0.0710392	ns
SQDG(32:1)	value	NL.Out	NO.In	5	5	2.1381	0.0325094	0.0710392	ns
PE(40:1)	value	NL.Out	NO.Out	5	5	-2.1201	0.0339934	0.0741675	ns
DGDG(38:7)	value	NL.Out	NO.Out	5	5	2.1130	0.0346048	0.0749570	ns
DGTS(34:2)	value	NL.In	NL.Out	5	5	-2.1122	0.0346729	0.0749570	ns
Cer(d32:1)	value	NL.Out	NO.Out	5	5	2.1122	0.0346729	0.0749570	ns
LPE(20:3)	value	NL.In	NL.Out	5	5	2.1122	0.0346729	0.0749570	ns
PC(37:5)	value	NL.Out	NO.Out	5	5	2.1122	0.0346729	0.0749570	ns
SQDG(30:1)	value	NL.Out	NO.Out	5	5	2.1122	0.0346729	0.0749570	ns
LPC(20:1)	value	NL.In	NL.Out	5	5	2.0972	0.0359780	0.0775416	ns
LPC(20:1)	value	NL.Out	NO.Out	5	5	-2.0972	0.0359780	0.0775416	ns
DGMG(16:0)	value	NL.In	NO.In	5	5	-2.0909	0.0365338	0.0782631	ns
PC(37:4)	value	NL.Out	NO.In	5	5	2.0909	0.0365338	0.0782631	ns
PE(32:1)	value	NL.In	NL.Out	5	5	2.0909	0.0365338	0.0782631	ns
PE(38:8)	value	NL.In	NO.In	5	5	-2.0917	0.0364629	0.0782631	ns
DGDG(36:7)	value	NL.In	NO.Out	5	5	-2.0846	0.0371022	0.0786478	ns
LPC(20:4)	value	NL.Out	NO.In	5	5	-2.0854	0.0370310	0.0786478	ns
MGTS(18:2)	value	NL.In	NO.Out	5	5	-2.0846	0.0371022	0.0786478	ns
PC(38:6)	value	NO.In	NO.Out	5	5	-2.0846	0.0371022	0.0786478	ns
PE(40:10)	value	NL.In	NO.In	5	5	2.0854	0.0370310	0.0786478	ns
PE(40:9)	value	NL.In	NL.Out	5	5	-2.0846	0.0371022	0.0786478	ns
PI(34:2)	value	NO.In	NO.Out	5	5	2.0846	0.0371022	0.0786478	ns

PE(34:4)	value	NO.In	NO.Out	5	5	2.0712	0.0383409	0.0811521	ns
PE(40:1)	value	NL.In	NO.In	5	5	2.0665	0.0387837	0.0819667	ns
PC(38:2)	value	NL.Out	NO.In	5	5	2.0641	0.0390055	0.0823127	ns
PE(36:5)	value	NL.Out	NO.Out	5	5	-2.0610	0.0393018	0.0828145	ns
PC(38:5)	value	NL.In	NL.Out	5	5	-2.0587	0.0395244	0.0831597	ns
LPE(16:3)	value	NO.In	NO.Out	5	5	2.0507	0.0402932	0.0846515	ns
PI(34:5)	value	NL.Out	NO.Out	5	5	2.0404	0.0413094	0.0866579	ns
DGMG(16:0)	value	NL.In	NO.Out	5	5	-2.0373	0.0416178	0.0871757	ns
PC(36:4(OH))	value	NL.In	NO.Out	5	5	-2.0366	0.0416950	0.0872084	ns
DGTS(36:5)	value	NL.In	NL.Out	5	5	2.0312	0.0422362	0.0873087	ns
DGTS(36:6)	value	NL.Out	NO.In	5	5	-2.0319	0.0421588	0.0873087	ns
DGTS(38:6)	value	NL.Out	NO.In	5	5	2.0312	0.0422362	0.0873087	ns
PE(40:9)	value	NL.Out	NO.Out	5	5	2.0312	0.0422362	0.0873087	ns
PI(32:2)	value	NL.In	NO.Out	5	5	2.0312	0.0422362	0.0873087	ns
PI(32:2)	value	NL.Out	NO.In	5	5	-2.0312	0.0422362	0.0873087	ns
PI(33:1)	value	NL.In	NL.Out	5	5	2.0342	0.0419267	0.0873087	ns
PI(34:1)	value	NL.Out	NO.Out	5	5	-2.0312	0.0422362	0.0873087	ns
PE(38:9)	value	NL.In	NO.Out	5	5	-2.0197	0.0434142	0.0896131	ns
PE(36:4)	value	NL.In	NL.Out	5	5	2.0075	0.0446985	0.0921297	ns
SQDG(30:1)	value	NL.In	NO.In	5	5	2.0052	0.0449402	0.0924933	ns
LPE(14:0)	value	NO.In	NO.Out	5	5	1.9994	0.0455690	0.0936513	ns
PG(31:1)	value	NL.In	NL.Out	5	5	1.9935	0.0462030	0.0948165	ns
PI(34:5)	value	NL.In	NO.Out	5	5	1.9867	0.0469539	0.0962180	ns
PE(38:8)	value	NL.Out	NO.Out	5	5	-1.9845	0.0472047	0.0965923	ns
DGTS(36:6)	value	NL.In	NL.Out	5	5	1.9785	0.0478749	0.0972609	ns
DGTS(38:5)	value	NL.Out	NO.In	5	5	1.9792	0.0477910	0.0972609	ns
PC(33:2)	value	NL.Out	NO.In	5	5	-1.9785	0.0478749	0.0972609	ns
PG(30:1)	value	NL.In	NL.Out	5	5	1.9785	0.0478749	0.0972609	ns
PG(32:1)	value	NL.Out	NO.Out	5	5	-1.9785	0.0478749	0.0972609	ns
DGTS(40:10)	value	NL.In	NO.Out	5	5	1.9777	0.0479588	0.0972918	ns
MGTS(16:4)	value	NL.In	NO.In	5	5	1.9704	0.0487947	0.0988459	ns
LPE(16:3)	value	NL.Out	NO.Out	5	5	-1.9676	0.0491149	0.0993524	ns
PC(37:2)	value	NL.In	NO.In	5	5	1.9584	0.0501851	0.1013724	ns
PC(38:2)	value	NO.In	NO.Out	5	5	-1.9569	0.0503591	0.1015790	ns
MGTS(18:5)	value	NL.In	NO.Out	5	5	1.9525	0.0508818	0.1023717	ns
PC(38:5)	value	NL.In	NO.Out	5	5	-1.9517	0.0509690	0.1023717	ns
SQDG(30:1)	value	NO.In	NO.Out	5	5	1.9517	0.0509690	0.1023717	ns
DGMG(14:0)	value	NL.In	NL.Out	5	5	1.9471	0.0515220	0.1028986	ns
DGMG(14:0)	value	NL.In	NO.In	5	5	-1.9471	0.0515220	0.1028986	ns
DGMG(14:0)	value	NL.Out	NO.Out	5	5	-1.9471	0.0515220	0.1028986	ns
DGMG(14:0)	value	NO.In	NO.Out	5	5	1.9471	0.0515220	0.1028986	ns
SQDG(32:3)	value	NL.In	NO.In	5	5	1.9456	0.0517017	0.1031121	ns
PG(32:2)	value	NL.In	NL.Out	5	5	1.9389	0.0525118	0.1044335	ns
PG(32:2)	value	NL.In	NO.Out	5	5	1.9389	0.0525118	0.1044335	ns
PI(40:10)	value	NL.Out	NO.In	5	5	1.9352	0.0529627	0.1051826	ns
PE(40:1)	value	NL.Out	NO.In	5	5	-1.9323	0.0533239	0.1057517	ns
DGTS(34:4)	value	NL.Out	NO.In	5	5	1.9250	0.0542288	0.1068281	ns
DGTS(36:8)	value	NL.In	NO.Out	5	5	1.9243	0.0543194	0.1068281	ns
LPC(16:0)	value	NL.In	NL.Out	5	5	1.9243	0.0543194	0.1068281	ns
PC(32:1)	value	NL.In	NO.Out	5	5	1.9243	0.0543194	0.1068281	ns
PC(38:8)	value	NL.In	NO.Out	5	5	1.9243	0.0543194	0.1068281	ns

PI(34:2)	value	NL.Out	NO.In	5	5	-1.9243	0.0543194	0.1068281	ns
PC(38:3)	value	NL.Out	NO.In	5	5	1.9149	0.0555039	0.1090062	ns
MGTS(16:4)	value	NL.Out	NO.Out	5	5	-1.9141	0.0556093	0.1090621	ns
PE(34:4)	value	NL.Out	NO.Out	5	5	-1.8829	0.0597138	0.1169498	ns
PC(31:1)	value	NL.In	NO.Out	5	5	1.8815	0.0599082	0.1171685	ns
MGTS(17:1)	value	NL.In	NL.Out	5	5	1.8779	0.0603946	0.1176324	ns
MGTS(17:1)	value	NO.In	NO.Out	5	5	-1.8779	0.0603946	0.1176324	ns
PE(40:1)	value	NL.In	NO.Out	5	5	1.8786	0.0602973	0.1176324	ns
PE(38:8)	value	NO.In	NO.Out	5	5	1.8772	0.0604919	0.1176601	ns
DGTS(34:5)	value	NL.In	NL.Out	5	5	1.8708	0.0613688	0.1180683	ns
DGTS(36:7)	value	NO.In	NO.Out	5	5	1.8722	0.0611738	0.1180683	ns
PC(36:4)	value	NL.In	NO.In	5	5	1.8708	0.0613688	0.1180683	ns
PE(36:7)	value	NL.In	NL.Out	5	5	1.8747	0.0608400	0.1180683	ns
PG(36:5)	value	NL.Out	NO.Out	5	5	-1.8708	0.0613688	0.1180683	ns
PI(32:2)	value	NL.In	NO.In	5	5	-1.8708	0.0613688	0.1180683	ns
PI(32:2)	value	NL.Out	NO.Out	5	5	1.8708	0.0613688	0.1180683	ns
PI(34:1)	value	NO.In	NO.Out	5	5	-1.8708	0.0613688	0.1180683	ns
PG(33:1)	value	NL.Out	NO.Out	5	5	1.8546	0.0636547	0.1223000	ns
DGDG(38:6)	value	NL.In	NO.Out	5	5	1.8462	0.0648652	0.1244568	ns
DGDG(38:7)	value	NO.In	NO.Out	5	5	-1.8455	0.0649662	0.1244819	ns
SQDG(30:1)	value	NL.In	NL.Out	5	5	1.8448	0.0650672	0.1245069	ns
PC(40:11)	value	NL.In	NO.In	5	5	-1.8256	0.0679059	0.1297634	ns
SQDG(28:0)	value	NO.In	NO.Out	5	5	1.8229	0.0683235	0.1303856	ns
PC(36:4(OH))	value	NL.In	NL.Out	5	5	-1.8222	0.0684280	0.1304092	ns
DGTS(34:3)	value	NL.In	NO.Out	5	5	1.8181	0.0690550	0.1305498	ns
PC(35:4)	value	NL.Out	NO.Out	5	5	-1.8194	0.0688459	0.1305498	ns
PC(40:10)	value	NL.In	NL.Out	5	5	1.8181	0.0690550	0.1305498	ns
PC(40:10)	value	NO.In	NO.Out	5	5	1.8181	0.0690550	0.1305498	ns
PE(36:5)	value	NO.In	NO.Out	5	5	1.8201	0.0687414	0.1305498	ns
PG(32:1)	value	NL.In	NO.Out	5	5	-1.8181	0.0690550	0.1305498	ns
DGTS(36:5)	value	NL.In	NO.Out	5	5	-1.8174	0.0691595	0.1305731	ns
PC(30:3)	value	NL.Out	NO.In	5	5	-1.8161	0.0693566	0.1307709	ns
PG(31:1)	value	NL.In	NO.Out	5	5	1.8050	0.0710815	0.1338449	ns
PC(32:5)	value	NL.In	NO.Out	5	5	1.7980	0.0721697	0.1357136	ns
PC(37:6)	value	NL.Out	NO.Out	5	5	1.7954	0.0725930	0.1363285	ns
DGMG(16:1)	value	NO.In	NO.Out	5	5	-1.7933	0.0729171	0.1367558	ns
DGTS(36:7)	value	NL.In	NO.In	5	5	1.7920	0.0731333	0.1369797	ns
DGTS(34:2)	value	NL.In	NO.In	5	5	1.7913	0.0732413	0.1370010	ns
LPC(20:0)	value	NL.In	NL.Out	5	5	-1.7801	0.0750665	0.1400450	ns
LPC(20:0)	value	NO.In	NO.Out	5	5	1.7801	0.0750665	0.1400450	ns
PC(40:11)	value	NL.Out	NO.Out	5	5	-1.7719	0.0764052	0.1423549	ns
DGDG(36:5)	value	NL.In	NO.In	5	5	-1.7639	0.0777447	0.1424147	ns
DGTS(40:9)	value	NL.In	NL.Out	5	5	1.7639	0.0777447	0.1424147	ns
LPE(18:2)	value	NL.Out	NO.Out	5	5	-1.7646	0.0776331	0.1424147	ns
MGDG(36:6)	value	NL.In	NL.Out	5	5	-1.7653	0.0775214	0.1424147	ns
MGDG(36:6)	value	NO.In	NO.Out	5	5	-1.7653	0.0775214	0.1424147	ns
PC(32:1)	value	NL.In	NL.Out	5	5	1.7639	0.0777447	0.1424147	ns
PC(33:3)	value	NL.In	NO.In	5	5	-1.7659	0.0774098	0.1424147	ns
PC(33:3)	value	NL.Out	NO.Out	5	5	1.7659	0.0774098	0.1424147	ns
PC(34:4)	value	NL.In	NO.Out	5	5	1.7639	0.0777447	0.1424147	ns
PC(36:4)	value	NL.In	NO.Out	5	5	-1.7639	0.0777447	0.1424147	ns

PC(38:7)	value	NO.In	NO.Out	5	5	1.7646	0.0776331	0.1424147	ns
PE(38:8)	value	NL.In	NL.Out	5	5	1.7699	0.0767400	0.1424147	ns
PI-Cer(d18:1/14:1)	value	NL.Out	NO.Out	5	5	1.7639	0.0777447	0.1424147	ns
DGTS(38:10)	value	NO.In	NO.Out	5	5	1.7491	0.0802782	0.1468655	ns
DGDG(38:7)	value	NL.In	NL.Out	5	5	-1.7385	0.0821217	0.1494834	ns
LPC(20:5)	value	NL.In	NO.In	5	5	-1.7379	0.0822370	0.1494834	ns
MGTS(18:5)	value	NO.In	NO.Out	5	5	-1.7385	0.0821217	0.1494834	ns
PE(36:7)	value	NL.In	NO.In	5	5	-1.7388	0.0820679	0.1494834	ns
SQDG(32:2)	value	NL.In	NO.Out	5	5	1.7379	0.0822370	0.1494834	ns
PE(34:4)	value	NL.In	NO.In	5	5	-1.7215	0.0851584	0.1545953	ns
LPC(18:5)	value	NL.In	NO.In	5	5	-1.7150	0.0863494	0.1559843	ns
LPC(18:5)	value	NL.Out	NO.Out	5	5	-1.7150	0.0863494	0.1559843	ns
MGDG(32:2)	value	NL.In	NO.In	5	5	-1.7137	0.0865845	0.1559843	ns
MGDG(32:2)	value	NL.Out	NO.Out	5	5	-1.7137	0.0865845	0.1559843	ns
PC(37:4)	value	NL.In	NO.Out	5	5	1.7156	0.0862280	0.1559843	ns
PE(38:7)	value	NL.In	NO.In	5	5	1.7143	0.0864657	0.1559843	ns
DGDG(36:5)	value	NO.In	NO.Out	5	5	-1.7105	0.0871786	0.1562594	ns
DGTS(36:5)	value	NO.In	NO.Out	5	5	-1.7105	0.0871786	0.1562594	ns
PC(34:4)	value	NL.In	NO.In	5	5	-1.7105	0.0871786	0.1562594	ns
PE(40:8)	value	NL.Out	NO.In	5	5	1.7105	0.0871786	0.1562594	ns
PE(36:3)	value	NL.In	NL.Out	5	5	1.7079	0.0876630	0.1567308	ns
PE(36:3)	value	NO.In	NO.Out	5	5	-1.7079	0.0876630	0.1567308	ns
LPE(16:3)	value	NL.In	NO.In	5	5	-1.6905	0.0909386	0.1623821	ns
DGMG(16:1)	value	NL.Out	NO.In	5	5	-1.6863	0.0917412	0.1630061	ns
PC(30:1)	value	NL.In	NO.In	5	5	-1.6856	0.0918636	0.1630061	ns
PC(30:1)	value	NL.Out	NO.Out	5	5	1.6856	0.0918636	0.1630061	ns
LPG(16:0)	value	NL.Out	NO.Out	5	5	1.6856	0.0918636	0.1630061	ns
SQDG(36:5)	value	NL.In	NO.Out	5	5	1.6863	0.0917412	0.1630061	ns
PC(36:7)	value	NL.In	NO.Out	5	5	1.6850	0.0919860	0.1630190	ns
LPE(18:2)	value	NL.In	NL.Out	5	5	-1.6844	0.0921084	0.1630319	ns
PE(32:3)	value	NL.In	NO.Out	5	5	1.6763	0.0936798	0.1656062	ns
PE(30:1)	value	NL.In	NO.Out	5	5	1.6728	0.0943635	0.1663994	ns
PE(30:1)	value	NL.Out	NO.Out	5	5	-1.6728	0.0943635	0.1663994	ns
DGDG(30:1)	value	NO.In	NO.Out	5	5	1.6583	0.0972636	0.1677788	ns
DGDG(35:5)	value	NL.Out	NO.In	5	5	1.6570	0.0975155	0.1677788	ns
DGTS(32:1)	value	NL.In	NO.Out	5	5	1.6570	0.0975155	0.1677788	ns
DGTS(32:5)	value	NL.Out	NO.In	5	5	-1.6677	0.0953729	0.1677788	ns
LPC(16:0)	value	NL.In	NO.In	5	5	-1.6570	0.0975155	0.1677788	ns
LPE(14:0)	value	NL.Out	NO.Out	5	5	-1.6614	0.0966245	0.1677788	ns
MGDG(40:8)	value	NL.In	NO.In	5	5	-1.6633	0.0962555	0.1677788	ns
MGDG(40:8)	value	NL.Out	NO.Out	5	5	-1.6633	0.0962555	0.1677788	ns
MGTS(16:3)	value	NO.In	NO.Out	5	5	1.6570	0.0975155	0.1677788	ns
MGTS(17:2)	value	NO.In	NO.Out	5	5	-1.6658	0.0957512	0.1677788	ns
MGTS(18:3)	value	NL.In	NO.In	5	5	1.6576	0.0973895	0.1677788	ns
MGTS(20:5)	value	NO.In	NO.Out	5	5	1.6570	0.0975155	0.1677788	ns
PC(32:2)	value	NL.In	NO.In	5	5	-1.6570	0.0975155	0.1677788	ns
PC(32:2)	value	NL.Out	NO.Out	5	5	1.6570	0.0975155	0.1677788	ns
PC(37:2)	value	NL.In	NO.Out	5	5	-1.6633	0.0962555	0.1677788	ns
PE(40:9)	value	NO.In	NO.Out	5	5	-1.6570	0.0975155	0.1677788	ns
PG(35:5)	value	NL.In	NO.In	5	5	1.6583	0.0972636	0.1677788	ns

PI(33:1)	value	NL.In	NO.In	5	5	1.6595	0.0970116	0.1677788	ns
PI-Cer(d18:1/14:1)	value	NL.In	NL.Out	5	5	1.6570	0.0975155	0.1677788	ns
SQDG(32:1)	value	NL.Out	NO.Out	5	5	1.6570	0.0975155	0.1677788	ns
PC(34:7)	value	NL.In	NO.Out	5	5	1.6560	0.0977181	0.1679233	ns
PE(30:0)	value	NO.In	NO.Out	5	5	-1.6501	0.0989163	0.1697763	ns
PE(34:3)	value	NO.In	NO.Out	5	5	1.6358	0.1018735	0.1746404	ns
LPE(18:3)	value	NO.In	NO.Out	5	5	1.6346	0.1021328	0.1748730	ns
PC(32:4)	value	NL.In	NL.Out	5	5	1.6334	0.1023919	0.1751050	ns
LPC(18:3)	value	NL.In	NO.Out	5	5	-1.6315	0.1027805	0.1753880	ns
PE(36:8)	value	NL.Out	NO.Out	5	5	-1.6314	0.1028051	0.1753880	ns
PI(34:3)	value	NL.In	NO.In	5	5	1.6164	0.1060124	0.1799922	ns
PI(34:3)	value	NL.Out	NO.Out	5	5	1.6164	0.1060124	0.1799922	ns
PI(36:6)	value	NL.In	NO.In	5	5	1.6170	0.1058790	0.1799922	ns
PI(36:6)	value	NL.Out	NO.Out	5	5	1.6170	0.1058790	0.1799922	ns
PG(31:0)	value	NL.In	NL.Out	5	5	1.6158	0.1061458	0.1800029	ns
PG(33:1)	value	NL.In	NL.Out	5	5	-1.6127	0.1068125	0.1809168	ns
DGTS(33:2)	value	NL.In	NO.Out	5	5	1.6054	0.1084104	0.1816912	ns
DGTS(36:4)	value	NL.Out	NO.Out	5	5	-1.6036	0.1088094	0.1816912	ns
LPC(20:4)	value	NO.In	NO.Out	5	5	-1.6042	0.1086764	0.1816912	ns
LPE(16:1)	value	NL.Out	NO.Out	5	5	-1.6036	0.1088094	0.1816912	ns
LPE(18:1)	value	NL.Out	NO.Out	5	5	-1.6036	0.1088094	0.1816912	ns
LPE(18:4)	value	NL.Out	NO.Out	5	5	-1.6066	0.1081443	0.1816912	ns
LPE(20:4)	value	NL.Out	NO.Out	5	5	-1.6036	0.1088094	0.1816912	ns
MGTS(14:0)	value	NO.In	NO.Out	5	5	1.6036	0.1088094	0.1816912	ns
MGTS(16:2)	value	NL.Out	NO.Out	5	5	1.6042	0.1086764	0.1816912	ns
MGTS(18:2)	value	NL.In	NO.In	5	5	1.6036	0.1088094	0.1816912	ns
PC(34:3)	value	NL.Out	NO.Out	5	5	1.6036	0.1088094	0.1816912	ns
PE(40:9)	value	NL.In	NO.In	5	5	1.6036	0.1088094	0.1816912	ns
PC(37:7)	value	NL.In	NO.Out	5	5	1.5822	0.1136031	0.1894723	ns
LPG(16:0)	value	NO.In	NO.Out	5	5	-1.5786	0.1144226	0.1906146	ns
PG(36:2)	value	NL.In	NL.Out	5	5	1.5780	0.1145591	0.1906178	ns
DGTS(34:2)	value	NL.Out	NO.Out	5	5	1.5774	0.1146956	0.1906208	ns
PC(31:2)	value	NL.In	NL.Out	5	5	1.5721	0.1159187	0.1924277	ns
PI(30:1)	value	NL.In	NO.In	5	5	1.5619	0.1183108	0.1959392	ns
PI(30:1)	value	NL.Out	NO.Out	5	5	1.5619	0.1183108	0.1959392	ns
MGTS(17:2)	value	NL.In	NL.Out	5	5	-1.5583	0.1191530	0.1971035	ns
DGTS(34:6)	value	NL.Out	NO.In	5	5	-1.5536	0.1202742	0.1984922	ns
DGTS(38:9)	value	NL.Out	NO.Out	5	5	1.5513	0.1208341	0.1984922	ns
LPE(20:5)	value	NL.In	NO.Out	5	5	-1.5501	0.1211139	0.1984922	ns
LPE(20:5)	value	NL.Out	NO.In	5	5	-1.5501	0.1211139	0.1984922	ns
PE(36:5)	value	NL.In	NL.Out	5	5	1.5525	0.1205542	0.1984922	ns
PG(30:1)	value	NL.In	NO.In	5	5	-1.5507	0.1209740	0.1984922	ns
PG(36:5(OH))	value	NL.In	NO.In	5	5	-1.5507	0.1209740	0.1984922	ns
PG(36:5(OH))	value	NL.Out	NO.Out	5	5	-1.5507	0.1209740	0.1984922	ns
DGTS(37:5)	value	NL.In	NO.Out	5	5	1.5286	0.1263711	0.2068688	ns
LPE(18:3)	value	NL.In	NL.Out	5	5	1.5274	0.1266581	0.2070992	ns
LPE(20:2)	value	NL.In	NO.In	5	5	1.5226	0.1278479	0.2080835	ns
LPE(20:2)	value	NL.Out	NO.In	5	5	1.5226	0.1278479	0.2080835	ns
PC(33:2)	value	NL.Out	NO.Out	5	5	1.5240	0.1275182	0.2080835	ns
PE(34:6)	value	NL.In	NO.In	5	5	-1.5230	0.1277684	0.2080835	ns

DGDG(30:1)	value	NL.In	NO.Out	5	5	-1.4978	0.1341879	0.2142014	ns
DGTS(30:3)	value	NL.Out	NO.In	5	5	-1.5052	0.1322791	0.2142014	ns
DGTS(36:4)	value	NL.In	NO.In	5	5	1.4967	0.1344810	0.2142014	ns
DGTS(38:9)	value	NL.In	NO.In	5	5	-1.4978	0.1341879	0.2142014	ns
Cer(d32:1)	value	NO.In	NO.Out	5	5	-1.4972	0.1343345	0.2142014	ns
LPC(18:2)	value	NL.Out	NO.In	5	5	-1.4972	0.1343345	0.2142014	ns
LPE(18:4)	value	NL.In	NO.In	5	5	-1.4995	0.1337481	0.2142014	ns
MGDG(34:5)	value	NL.In	NO.In	5	5	-1.4972	0.1343345	0.2142014	ns
MGDG(34:5)	value	NL.Out	NO.Out	5	5	-1.4972	0.1343345	0.2142014	ns
MGTS(15:0)	value	NL.In	NO.Out	5	5	1.4989	0.1338947	0.2142014	ns
MGTS(15:0)	value	NL.Out	NO.In	5	5	-1.4989	0.1338947	0.2142014	ns
MGTS(18:4)	value	NL.In	NL.Out	5	5	1.4967	0.1344810	0.2142014	ns
PC(34:1)	value	NL.In	NO.In	5	5	1.4967	0.1344810	0.2142014	ns
PC(34:1)	value	NL.Out	NO.Out	5	5	-1.4967	0.1344810	0.2142014	ns
PC(38:1)	value	NL.Out	NO.In	5	5	1.5001	0.1336014	0.2142014	ns
PE(32:2)	value	NL.In	NO.Out	5	5	1.4972	0.1343345	0.2142014	ns
LPG(16:0)	value	NL.In	NO.Out	5	5	1.4984	0.1340414	0.2142014	ns
SQDG(31:1)	value	NL.Out	NO.Out	5	5	1.4967	0.1344810	0.2142014	ns
SQDG(34:2)	value	NL.In	NO.Out	5	5	1.4989	0.1338947	0.2142014	ns
SQDG(28:0)	value	NL.Out	NO.Out	5	5	-1.4744	0.1403799	0.2233460	ns
DGTS(32:3)	value	NL.Out	NO.In	5	5	1.4710	0.1412794	0.2235086	ns
PC(33:2)	value	NL.In	NL.Out	5	5	1.4705	0.1414292	0.2235086	ns
PC(37:5)	value	NL.Out	NO.In	5	5	1.4705	0.1414292	0.2235086	ns
PE(38:7)	value	NL.In	NO.Out	5	5	-1.4733	0.1406799	0.2235086	ns
PG(35:5)	value	NL.In	NL.Out	5	5	-1.4710	0.1412794	0.2235086	ns
SQDG(36:5)	value	NL.Out	NO.In	5	5	-1.4722	0.1409798	0.2235086	ns
LPE(14:0)	value	NL.In	NO.Out	5	5	1.4643	0.1431059	0.2259062	ns
PC(30:3)	value	NL.In	NO.In	5	5	1.4637	0.1432720	0.2259167	ns
LPE(16:2)	value	NO.In	NO.Out	5	5	1.4575	0.1449708	0.2283411	ns
MGDG(30:1)	value	NL.In	NO.In	5	5	-1.4547	0.1457409	0.2290446	ns
MGDG(30:1)	value	NL.Out	NO.Out	5	5	-1.4547	0.1457409	0.2290446	ns
DGDG(30:1)	value	NL.Out	NO.Out	5	5	1.4443	0.1486554	0.2300207	ns
DGDG(32:1)	value	NL.Out	NO.In	5	5	1.4432	0.1489611	0.2300207	ns
DGDG(34:5)	value	NL.Out	NO.Out	5	5	1.4432	0.1489611	0.2300207	ns
DGDG(36:6)	value	NL.Out	NO.Out	5	5	1.4432	0.1489611	0.2300207	ns
DGTS(28:0)	value	NL.Out	NO.In	5	5	-1.4443	0.1486554	0.2300207	ns
DGTS(33:1)	value	NO.In	NO.Out	5	5	-1.4481	0.1475839	0.2300207	ns
DGTS(38:8)	value	NL.In	NL.Out	5	5	1.4432	0.1489611	0.2300207	ns
LPE(20:4)	value	NL.In	NO.Out	5	5	-1.4432	0.1489611	0.2300207	ns
LPE(20:4)	value	NO.In	NO.Out	5	5	1.4432	0.1489611	0.2300207	ns
MGDG(40:10)	value	NL.Out	NO.In	5	5	-1.4432	0.1489611	0.2300207	ns
PC(32:1)	value	NL.In	NO.In	5	5	-1.4432	0.1489611	0.2300207	ns
PC(32:3)	value	NL.In	NO.In	5	5	-1.4432	0.1489611	0.2300207	ns
PC(32:3)	value	NL.Out	NO.Out	5	5	1.4432	0.1489611	0.2300207	ns
PC(38:10)	value	NL.In	NO.In	5	5	-1.4492	0.1472772	0.2300207	ns
PE(40:10)	value	NL.In	NO.Out	5	5	-1.4438	0.1488083	0.2300207	ns
PI(32:1)	value	NL.In	NO.Out	5	5	1.4432	0.1489611	0.2300207	ns
DGDG(30:0)	value	NL.In	NO.In	5	5	1.3908	0.1642847	0.2350705	ns
DGDG(30:0)	value	NL.In	NO.Out	5	5	-1.4176	0.1563219	0.2350705	ns
DGDG(32:1)	value	NL.Out	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
DGDG(34:1)	value	NL.In	NL.Out	5	5	-1.3373	0.1811208	0.2350705	ns

DGDG(34:1)	value	NL.In	NO.In	5	5	1.3373	0.1811208	0.2350705	ns
DGDG(34:1)	value	NL.Out	NO.Out	5	5	-1.3373	0.1811208	0.2350705	ns
DGDG(34:2)	value	NL.In	NL.Out	5	5	-1.4170	0.1564778	0.2350705	ns
DGDG(34:2)	value	NL.In	NO.In	5	5	1.3368	0.1812850	0.2350705	ns
DGDG(34:3)	value	NL.In	NO.In	5	5	1.3388	0.1806276	0.2350705	ns
DGDG(34:3)	value	NL.In	NO.Out	5	5	-1.3388	0.1806276	0.2350705	ns
DGDG(34:3)	value	NL.Out	NO.Out	5	5	1.3388	0.1806276	0.2350705	ns
DGDG(35:5)	value	NL.Out	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
DGDG(36:7)	value	NL.In	NO.In	5	5	1.3898	0.1646022	0.2350705	ns
DGDG(40:10)	value	NL.In	NO.Out	5	5	1.3898	0.1646022	0.2350705	ns
DGDG(40:10)	value	NL.Out	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
DGTS(28:0)	value	NL.Out	NO.Out	5	5	1.3373	0.1811208	0.2350705	ns
DGTS(28:1)	value	NL.In	NL.Out	5	5	1.3460	0.1783171	0.2350705	ns
DGTS(28:1)	value	NL.Out	NO.In	5	5	1.3460	0.1783171	0.2350705	ns
DGTS(28:1)	value	NO.In	NO.Out	5	5	1.3460	0.1783171	0.2350705	ns
DGTS(30:1)	value	NL.In	NL.Out	5	5	1.3363	0.1814492	0.2350705	ns
DGTS(30:1)	value	NL.Out	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
DGTS(30:1)	value	NO.In	NO.Out	5	5	1.3363	0.1814492	0.2350705	ns
DGTS(30:2)	value	NL.In	NL.Out	5	5	1.3403	0.1801337	0.2350705	ns
DGTS(30:2)	value	NL.Out	NO.In	5	5	1.3403	0.1801337	0.2350705	ns
DGTS(30:2)	value	NO.In	NO.Out	5	5	1.3403	0.1801337	0.2350705	ns
DGTS(30:3)	value	NL.Out	NO.Out	5	5	1.3439	0.1789787	0.2350705	ns
DGTS(32:2)	value	NL.Out	NO.In	5	5	1.3898	0.1646022	0.2350705	ns
DGTS(32:2)	value	NO.In	NO.Out	5	5	1.3363	0.1814492	0.2350705	ns
DGTS(32:3)	value	NO.In	NO.Out	5	5	1.3373	0.1811208	0.2350705	ns
DGTS(32:4)	value	NL.In	NL.Out	5	5	1.3363	0.1814492	0.2350705	ns
DGTS(32:4)	value	NL.Out	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
DGTS(32:4)	value	NO.In	NO.Out	5	5	1.3363	0.1814492	0.2350705	ns
DGTS(32:5)	value	NL.Out	NO.Out	5	5	1.3987	0.1618950	0.2350705	ns
DGTS(33:1)	value	NL.Out	NO.Out	5	5	1.3677	0.1714161	0.2350705	ns
DGTS(33:2)	value	NO.In	NO.Out	5	5	-1.3378	0.1809565	0.2350705	ns
DGTS(34:1)	value	NL.In	NL.Out	5	5	-1.3368	0.1812850	0.2350705	ns
DGTS(34:1)	value	NL.In	NO.In	5	5	1.3368	0.1812850	0.2350705	ns
DGTS(34:1)	value	NL.Out	NO.Out	5	5	-1.3368	0.1812850	0.2350705	ns
DGTS(34:3)	value	NL.In	NL.Out	5	5	-1.3368	0.1812850	0.2350705	ns
DGTS(34:6)	value	NL.Out	NO.Out	5	5	1.3393	0.1804630	0.2350705	ns
DGTS(36:2)	value	NL.In	NO.Out	5	5	-1.3771	0.1684869	0.2350705	ns
DGTS(36:2)	value	NL.Out	NO.In	5	5	-1.3771	0.1684869	0.2350705	ns
DGTS(36:6)	value	NL.Out	NO.Out	5	5	1.3368	0.1812850	0.2350705	ns
DGTS(38:8)	value	NO.In	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
DGTS(40:9)	value	NL.Out	NO.Out	5	5	1.3363	0.1814492	0.2350705	ns
Cer(d32:2)	value	NL.In	NL.Out	5	5	-1.3368	0.1812850	0.2350705	ns
Cer(d32:2)	value	NL.Out	NO.Out	5	5	-1.3368	0.1812850	0.2350705	ns
Cer(d32:2)	value	NO.In	NO.Out	5	5	1.3368	0.1812850	0.2350705	ns
Cer(d32:1)	value	NL.In	NO.Out	5	5	1.4170	0.1564778	0.2350705	ns
LPC(14:0)	value	NL.In	NO.Out	5	5	1.3945	0.1631713	0.2350705	ns
LPC(14:0)	value	NL.Out	NO.Out	5	5	-1.3409	0.1799690	0.2350705	ns
LPC(16:1)	value	NL.In	NO.In	5	5	-1.3363	0.1814492	0.2350705	ns
LPC(16:1)	value	NL.In	NO.Out	5	5	1.3363	0.1814492	0.2350705	ns
LPC(16:1)	value	NL.Out	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
LPC(16:2)	value	NL.In	NO.In	5	5	-1.3908	0.1642847	0.2350705	ns

LPC(16:2)	value	NL.Out	NO.Out	5	5	-1.3908	0.1642847	0.2350705	ns
LPC(16:3)	value	NL.Out	NO.Out	5	5	-1.4305	0.1525632	0.2350705	ns
LPC(17:0)	value	NL.In	NO.In	5	5	-1.3686	0.1711286	0.2350705	ns
LPC(17:0)	value	NL.In	NO.Out	5	5	1.3686	0.1711286	0.2350705	ns
LPC(17:0)	value	NL.Out	NO.Out	5	5	-1.3686	0.1711286	0.2350705	ns
LPC(18:1)	value	NL.Out	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
LPC(18:1)	value	NL.Out	NO.Out	5	5	-1.3898	0.1646022	0.2350705	ns
LPC(18:2)	value	NO.In	NO.Out	5	5	-1.3368	0.1812850	0.2350705	ns
LPC(18:3)	value	NL.In	NL.Out	5	5	1.3373	0.1811208	0.2350705	ns
LPC(18:4)	value	NL.In	NO.In	5	5	-1.3363	0.1814492	0.2350705	ns
LPC(18:4)	value	NL.In	NO.Out	5	5	1.3363	0.1814492	0.2350705	ns
LPC(18:4)	value	NL.Out	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
LPC(20:1)	value	NL.Out	NO.In	5	5	1.3981	0.1620778	0.2350705	ns
LPC(20:3)	value	NL.In	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
LPC(20:3)	value	NL.Out	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
LPC(20:3)	value	NO.In	NO.Out	5	5	1.3363	0.1814492	0.2350705	ns
LPC(20:5)	value	NL.In	NL.Out	5	5	1.3368	0.1812850	0.2350705	ns
LPC(22:6)	value	NL.In	NO.In	5	5	1.3419	0.1796392	0.2350705	ns
LPC(22:6)	value	NL.Out	NO.Out	5	5	-1.3419	0.1796392	0.2350705	ns
LPC(22:6)	value	NO.In	NO.Out	5	5	1.3419	0.1796392	0.2350705	ns
LPE(16:0)	value	NL.In	NL.Out	5	5	1.3687	0.1710918	0.2350705	ns
LPE(16:0)	value	NO.In	NO.Out	5	5	-1.3687	0.1710918	0.2350705	ns
LPE(16:1)	value	NL.In	NO.In	5	5	-1.3898	0.1646022	0.2350705	ns
LPE(16:2)	value	NL.In	NO.Out	5	5	-1.4035	0.1604544	0.2350705	ns
MGDG(32:1)	value	NL.In	NO.In	5	5	-1.3373	0.1811208	0.2350705	ns
MGDG(32:1)	value	NL.Out	NO.In	5	5	1.3641	0.1725492	0.2350705	ns
MGDG(32:5)	value	NL.In	NL.Out	5	5	-1.3465	0.1781515	0.2350705	ns
MGDG(32:5)	value	NL.Out	NO.In	5	5	-1.3465	0.1781515	0.2350705	ns
MGDG(32:5)	value	NO.In	NO.Out	5	5	-1.3465	0.1781515	0.2350705	ns
MGDG(36:5)	value	NL.In	NO.In	5	5	-1.3908	0.1642847	0.2350705	ns
MGDG(36:5)	value	NL.Out	NO.Out	5	5	-1.3908	0.1642847	0.2350705	ns
MGDG(38:7)	value	NL.In	NO.In	5	5	-1.3465	0.1781515	0.2350705	ns
MGDG(38:7)	value	NL.Out	NO.In	5	5	1.3465	0.1781515	0.2350705	ns
MGDG(38:7)	value	NL.Out	NO.Out	5	5	-1.3465	0.1781515	0.2350705	ns
MGDG(40:10)	value	NO.In	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
MGDG(40:9)	value	NL.In	NL.Out	5	5	-1.3368	0.1812850	0.2350705	ns
MGDG(40:9)	value	NL.Out	NO.In	5	5	-1.3368	0.1812850	0.2350705	ns
MGDG(40:9)	value	NO.In	NO.Out	5	5	-1.3368	0.1812850	0.2350705	ns
MGMG(16:0)	value	NL.In	NO.In	5	5	-1.3388	0.1806276	0.2350705	ns
MGMG(16:0)	value	NL.Out	NO.In	5	5	1.3924	0.1638079	0.2350705	ns
MGTS(14:0)	value	NL.In	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
MGTS(16:0)	value	NL.In	NO.Out	5	5	-1.3898	0.1646022	0.2350705	ns
MGTS(16:0)	value	NL.Out	NO.In	5	5	-1.3898	0.1646022	0.2350705	ns
MGTS(16:1)	value	NL.In	NL.Out	5	5	1.3635	0.1727108	0.2350705	ns
MGTS(16:1)	value	NL.Out	NO.In	5	5	1.3368	0.1812850	0.2350705	ns
MGTS(16:2)	value	NL.In	NL.Out	5	5	1.3368	0.1812850	0.2350705	ns
MGTS(16:3)	value	NL.In	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
MGTS(18:1)	value	NL.In	NL.Out	5	5	-1.3363	0.1814492	0.2350705	ns
MGTS(18:1)	value	NL.In	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
MGTS(18:1)	value	NL.Out	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
MGTS(18:3)	value	NL.In	NO.Out	5	5	-1.3368	0.1812850	0.2350705	ns

MGTS(20:5)	value	NL.Out	NO.Out	5	5	-1.3898	0.1646022	0.2350705	ns
PC(32:4)	value	NL.In	NO.In	5	5	-1.3388	0.1806276	0.2350705	ns
PC(34:5)	value	NL.In	NL.Out	5	5	1.3363	0.1814492	0.2350705	ns
PC(34:5)	value	NL.In	NO.In	5	5	-1.3363	0.1814492	0.2350705	ns
PC(34:5)	value	NL.Out	NO.Out	5	5	1.3363	0.1814492	0.2350705	ns
PC(34:6)	value	NL.In	NO.In	5	5	-1.3661	0.1719022	0.2350705	ns
PC(34:6)	value	NL.Out	NO.Out	5	5	1.3661	0.1719022	0.2350705	ns
PC(34:7)	value	NL.In	NO.In	5	5	-1.3574	0.1746566	0.2350705	ns
PC(35:2)	value	NL.In	NL.Out	5	5	-1.3635	0.1727108	0.2350705	ns
PC(35:2)	value	NL.In	NO.In	5	5	1.3368	0.1812850	0.2350705	ns
PC(36:2)	value	NL.In	NL.Out	5	5	-1.3363	0.1814492	0.2350705	ns
PC(36:2)	value	NL.In	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
PC(36:2)	value	NL.Out	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
PC(36:4(OH))	value	NL.In	NO.In	5	5	1.3934	0.1634897	0.2350705	ns
PC(36:5)	value	NL.In	NO.Out	5	5	1.3898	0.1646022	0.2350705	ns
PC(36:6)	value	NL.In	NL.Out	5	5	1.3635	0.1727108	0.2350705	ns
PC(36:6)	value	NL.In	NO.In	5	5	-1.3903	0.1644435	0.2350705	ns
PC(36:7)	value	NL.In	NO.In	5	5	-1.3908	0.1642847	0.2350705	ns
PC(36:8)	value	NL.In	NO.In	5	5	-1.3388	0.1806276	0.2350705	ns
PC(36:8)	value	NL.In	NO.Out	5	5	1.3388	0.1806276	0.2350705	ns
PC(36:8)	value	NL.Out	NO.Out	5	5	-1.3388	0.1806276	0.2350705	ns
PC(37:3)	value	NL.In	NL.Out	5	5	-1.3414	0.1798041	0.2350705	ns
PC(37:5)	value	NL.In	NL.Out	5	5	1.3368	0.1812850	0.2350705	ns
PC(38:1)	value	NL.Out	NO.Out	5	5	-1.3393	0.1804630	0.2350705	ns
PC(38:10)	value	NL.Out	NO.Out	5	5	-1.3419	0.1796392	0.2350705	ns
PC(38:2)	value	NL.In	NO.In	5	5	-1.3403	0.1801337	0.2350705	ns
PC(38:5)	value	NL.In	NO.In	5	5	1.3368	0.1812850	0.2350705	ns
PC(38:9)	value	NL.In	NL.Out	5	5	1.3898	0.1646022	0.2350705	ns
PC(38:9)	value	NL.In	NO.In	5	5	-1.3363	0.1814492	0.2350705	ns
PC(40:5)	value	NL.In	NO.In	5	5	-1.3424	0.1794742	0.2350705	ns
PC(40:5)	value	NL.Out	NO.In	5	5	1.3961	0.1626932	0.2350705	ns
PC(40:8)	value	NL.In	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
PC(40:8)	value	NL.In	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
PC(40:8)	value	NL.Out	NO.Out	5	5	1.3363	0.1814492	0.2350705	ns
PE(30:1)	value	NO.In	NO.Out	5	5	1.4030	0.1606147	0.2350705	ns
PE(30:3)	value	NL.In	NL.Out	5	5	-1.3955	0.1628526	0.2350705	ns
PE(32:2)	value	NL.Out	NO.Out	5	5	-1.3368	0.1812850	0.2350705	ns
PE(34:2)	value	NL.In	NO.In	5	5	-1.3368	0.1812850	0.2350705	ns
PE(34:2)	value	NL.In	NO.Out	5	5	1.3368	0.1812850	0.2350705	ns
PE(34:2)	value	NL.Out	NO.Out	5	5	-1.3368	0.1812850	0.2350705	ns
PE(34:5)	value	NL.In	NO.Out	5	5	1.3934	0.1634897	0.2350705	ns
PE(34:5)	value	NL.Out	NO.Out	5	5	-1.3398	0.1802984	0.2350705	ns
PE(34:6)	value	NL.Out	NO.Out	5	5	-1.3568	0.1748384	0.2350705	ns
PE(36:2)	value	NL.In	NL.Out	5	5	1.3590	0.1741547	0.2350705	ns
PE(36:2)	value	NL.In	NO.Out	5	5	-1.3590	0.1741547	0.2350705	ns
PE(36:2)	value	NO.In	NO.Out	5	5	1.3590	0.1741547	0.2350705	ns
PE(36:8)	value	NL.In	NO.In	5	5	-1.3595	0.1739872	0.2350705	ns
PE(38:5)	value	NL.In	NL.Out	5	5	-1.3388	0.1806276	0.2350705	ns
PE(38:5)	value	NL.In	NO.In	5	5	1.3388	0.1806276	0.2350705	ns
PE(38:5)	value	NL.Out	NO.Out	5	5	-1.3388	0.1806276	0.2350705	ns
PE(38:6)	value	NL.In	NL.Out	5	5	-1.3363	0.1814492	0.2350705	ns

PE(38:6)	value	NL.In	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
PE(38:6)	value	NL.Out	NO.Out	5	5	-1.3363	0.1814492	0.2350705	ns
PE(38:7)	value	NL.In	NL.Out	5	5	-1.4197	0.1556978	0.2350705	ns
PE(40:8)	value	NL.In	NO.Out	5	5	1.3898	0.1646022	0.2350705	ns
PG(30:1)	value	NL.In	NO.Out	5	5	1.3903	0.1644435	0.2350705	ns
PG(31:0)	value	NL.In	NO.In	5	5	-1.4003	0.1614154	0.2350705	ns
PG(31:1)	value	NL.In	NO.In	5	5	-1.4278	0.1533490	0.2350705	ns
PG(32:1)	value	NO.In	NO.Out	5	5	1.3368	0.1812850	0.2350705	ns
PG(32:2)	value	NL.In	NO.In	5	5	-1.4003	0.1614154	0.2350705	ns
PG(33:1)	value	NL.Out	NO.In	5	5	-1.4245	0.1542901	0.2350705	ns
PG(34:1)	value	NL.Out	NO.In	5	5	1.3363	0.1814492	0.2350705	ns
PG(34:5)	value	NL.In	NL.Out	5	5	1.3903	0.1644435	0.2350705	ns
PG(34:5)	value	NL.Out	NO.Out	5	5	1.3635	0.1727108	0.2350705	ns
PI(34:5)	value	NO.In	NO.Out	5	5	-1.3424	0.1794742	0.2350705	ns
PI-Cer(d18:1/14:0)	value	NL.In	NL.Out	5	5	-1.3465	0.1781515	0.2350705	ns
PI-Cer(d18:1/14:0)	value	NL.In	NO.Out	5	5	1.3465	0.1781515	0.2350705	ns
PI-Cer(d18:1/14:0)	value	NO.In	NO.Out	5	5	-1.3465	0.1781515	0.2350705	ns
SQDG(32:1)	value	NL.In	NL.Out	5	5	1.3363	0.1814492	0.2350705	ns
SQDG(34:2)	value	NO.In	NO.Out	5	5	-1.3383	0.1807921	0.2350705	ns
PC(31:2)	value	NL.Out	NO.Out	5	5	1.3282	0.1841169	0.2383086	ns
LPC(16:3)	value	NL.In	NO.In	5	5	-1.3226	0.1859780	0.2404976	ns
DGTS(38:10)	value	NL.Out	NO.Out	5	5	-1.3185	0.1873253	0.2420189	ns
PC(37:3)	value	NL.Out	NO.Out	5	5	-1.3145	0.1886675	0.2435307	ns
LPE(20:2)	value	NO.In	NO.Out	5	5	1.3051	0.1918509	0.2474143	ns
DGTS(36:2)	value	NL.In	NO.In	5	5	1.2961	0.1949489	0.2511808	ns
LPE(16:0)	value	NL.In	NO.In	5	5	-1.2882	0.1976791	0.2544669	ns
PC(34:6)	value	NL.In	NL.Out	5	5	1.2858	0.1985277	0.2550955	ns
PE(36:6)	value	NL.In	NO.Out	5	5	-1.2858	0.1985277	0.2550955	ns
DGDG(34:5)	value	NL.Out	NO.In	5	5	-1.2829	0.1995432	0.2554731	ns
DGDG(36:6)	value	NL.Out	NO.In	5	5	-1.2829	0.1995432	0.2554731	ns
MGDG(32:1)	value	NL.Out	NO.Out	5	5	-1.2838	0.1992051	0.2554731	ns
PC(35:2)	value	NL.Out	NO.Out	5	5	-1.2833	0.1993742	0.2554731	ns
DGTS(37:5)	value	NO.In	NO.Out	5	5	-1.2604	0.2075253	0.2654524	ns
PE(36:6)	value	NL.In	NL.Out	5	5	1.2590	0.2080409	0.2658717	ns
MGTS(16:1)	value	NO.In	NO.Out	5	5	1.2566	0.2088984	0.2664866	ns
PG(36:2)	value	NO.In	NO.Out	5	5	-1.2571	0.2087271	0.2664866	ns
LPC(16:3)	value	NL.In	NO.Out	5	5	1.2416	0.2143851	0.2732397	ns
DGDG(34:5)	value	NL.In	NL.Out	5	5	-1.2294	0.2189212	0.2736032	ns
DGDG(40:10)	value	NL.In	NO.In	5	5	-1.2294	0.2189212	0.2736032	ns
DGMG(16:0)	value	NL.In	NL.Out	5	5	1.2331	0.2175325	0.2736032	ns
DGTS(32:1)	value	NO.In	NO.Out	5	5	-1.2294	0.2189212	0.2736032	ns
DGTS(32:2)	value	NL.In	NL.Out	5	5	1.2294	0.2189212	0.2736032	ns
DGTS(36:8)	value	NL.In	NO.In	5	5	-1.2294	0.2189212	0.2736032	ns
LPC(14:0)	value	NL.In	NO.In	5	5	-1.2336	0.2173585	0.2736032	ns
LPC(16:2)	value	NL.In	NO.Out	5	5	1.2303	0.2185746	0.2736032	ns
MGDG(36:5)	value	NL.Out	NO.In	5	5	1.2303	0.2185746	0.2736032	ns
MGMG(16:0)	value	NL.Out	NO.Out	5	5	-1.2317	0.2180540	0.2736032	ns
MGTS(16:0)	value	NL.In	NO.In	5	5	1.2294	0.2189212	0.2736032	ns
PC(36:5)	value	NL.In	NO.In	5	5	-1.2294	0.2189212	0.2736032	ns

PC(38:7)	value	NL.Out	NO.Out	5	5	-1.2299	0.2187480	0.2736032	ns
PC(38:9)	value	NL.Out	NO.Out	5	5	1.2294	0.2189212	0.2736032	ns
PC(40:5)	value	NL.Out	NO.Out	5	5	-1.2350	0.2168358	0.2736032	ns
PE(30:3)	value	NO.In	NO.Out	5	5	-1.2345	0.2170101	0.2736032	ns
PE(32:1)	value	NL.In	NO.Out	5	5	-1.2331	0.2175325	0.2736032	ns
PE(34:5)	value	NL.In	NO.In	5	5	-1.2326	0.2177064	0.2736032	ns
PG(34:1)	value	NL.In	NO.In	5	5	-1.2294	0.2189212	0.2736032	ns
PI(32:1)	value	NL.Out	NO.Out	5	5	-1.2294	0.2189212	0.2736032	ns
PI(34:1)	value	NL.In	NO.Out	5	5	1.2294	0.2189212	0.2736032	ns
SQDG(31:1)	value	NL.In	NL.Out	5	5	1.2294	0.2189212	0.2736032	ns
PC(37:3)	value	NL.In	NO.In	5	5	1.2072	0.2273464	0.2838823	ns
PC(37:4)	value	NO.In	NO.Out	5	5	-1.2063	0.2276983	0.2840712	ns
DGMG(20:5)	value	NO.In	NO.Out	5	5	1.1881	0.2348056	0.2926802	ns
PC(38:10)	value	NL.In	NO.Out	5	5	1.1808	0.2376652	0.2959841	ns
DGDG(34:2)	value	NL.Out	NO.Out	5	5	-1.1764	0.2394383	0.2960689	ns
DGDG(36:7)	value	NL.Out	NO.Out	5	5	-1.1759	0.2396151	0.2960689	ns
DGMG(16:1)	value	NL.In	NO.In	5	5	1.1777	0.2389075	0.2960689	ns
DGTS(36:7)	value	NL.In	NL.Out	5	5	1.1768	0.2392615	0.2960689	ns
LPC(18:1)	value	NL.In	NO.In	5	5	-1.1759	0.2396151	0.2960689	ns
MGTS(18:4)	value	NL.Out	NO.In	5	5	1.1759	0.2396151	0.2960689	ns
MGTS(18:4)	value	NO.In	NO.Out	5	5	1.1759	0.2396151	0.2960689	ns
PC(37:6)	value	NL.In	NL.Out	5	5	1.1791	0.2383757	0.2960689	ns
PG(36:5)	value	NL.In	NL.Out	5	5	-1.1759	0.2396151	0.2960689	ns
PC(30:3)	value	NO.In	NO.Out	5	5	1.1656	0.2437963	0.3009726	ns
PG(34:5)	value	NL.In	NO.In	5	5	-1.1497	0.2502857	0.3087148	ns
DGDG(32:1)	value	NL.In	NO.In	5	5	-1.1225	0.2616511	0.3163945	ns
DGDG(36:5)	value	NL.Out	NO.In	5	5	-1.1225	0.2616511	0.3163945	ns
DGDG(36:6)	value	NL.In	NO.In	5	5	1.1225	0.2616511	0.3163945	ns
DGDG(38:6)	value	NO.In	NO.Out	5	5	-1.1238	0.2611126	0.3163945	ns
DGTS(28:0)	value	NL.In	NO.In	5	5	1.1233	0.2612922	0.3163945	ns
DGTS(34:4)	value	NO.In	NO.Out	5	5	1.1229	0.2614717	0.3163945	ns
DGTS(34:5)	value	NL.Out	NO.Out	5	5	1.1225	0.2616511	0.3163945	ns
DGTS(38:6)	value	NL.In	NO.In	5	5	-1.1225	0.2616511	0.3163945	ns
DGTS(40:10)	value	NL.In	NO.In	5	5	-1.1225	0.2616511	0.3163945	ns
LPC(20:4)	value	NL.In	NO.In	5	5	-1.1229	0.2614717	0.3163945	ns
MGDG(30:1)	value	NL.Out	NO.In	5	5	1.1315	0.2578593	0.3163945	ns
MGDG(40:10)	value	NL.In	NL.Out	5	5	-1.1225	0.2616511	0.3163945	ns
MGTS(18:2)	value	NL.In	NL.Out	5	5	-1.1225	0.2616511	0.3163945	ns
PC(32:3)	value	NL.In	NL.Out	5	5	1.1225	0.2616511	0.3163945	ns
PC(34:3)	value	NL.In	NO.Out	5	5	-1.1225	0.2616511	0.3163945	ns
PC(34:4)	value	NL.In	NL.Out	5	5	1.1225	0.2616511	0.3163945	ns
PC(36:4)	value	NL.Out	NO.Out	5	5	-1.1225	0.2616511	0.3163945	ns
PC(36:5)	value	NL.Out	NO.Out	5	5	-1.1225	0.2616511	0.3163945	ns
PC(36:6)	value	NL.Out	NO.Out	5	5	1.1229	0.2614717	0.3163945	ns
PE(32:3)	value	NL.In	NO.In	5	5	-1.1267	0.2598726	0.3163945	ns
PE(40:10)	value	NL.Out	NO.Out	5	5	-1.1229	0.2614717	0.3163945	ns
PI(34:2)	value	NL.In	NO.In	5	5	1.1225	0.2616511	0.3163945	ns
SQDG(31:1)	value	NO.In	NO.Out	5	5	-1.1225	0.2616511	0.3163945	ns
PC(37:2)	value	NL.In	NL.Out	5	5	1.0999	0.2713696	0.3272480	ns
PE(30:0)	value	NL.In	NL.Out	5	5	1.1001	0.2712943	0.3272480	ns
PE(30:0)	value	NL.In	NO.In	5	5	-1.1001	0.2712943	0.3272480	ns

PE(34:3)	value	NL.Out	NO.Out	5	5	-1.0995	0.2715511	0.3272480	ns
MGTS(18:5)	value	NL.In	NL.Out	5	5	1.0966	0.2728181	0.3284952	ns
PC(32:5)	value	NL.In	NO.In	5	5	-1.0897	0.2758344	0.3318450	ns
DGTS(38:10)	value	NL.In	NO.Out	5	5	-1.0764	0.2817692	0.3386972	ns
PC(37:7)	value	NL.In	NO.In	5	5	-1.0727	0.2834145	0.3403859	ns
DGTS(32:3)	value	NL.In	NL.Out	5	5	1.0698	0.2846870	0.3406160	ns
LPC(16:0)	value	NO.In	NO.Out	5	5	1.0690	0.2850494	0.3406160	ns
LPE(18:1)	value	NL.In	NL.Out	5	5	-1.0690	0.2850494	0.3406160	ns
LPE(18:1)	value	NL.In	NO.In	5	5	1.0690	0.2850494	0.3406160	ns
PE(36:4)	value	NL.In	NO.In	5	5	-1.0707	0.2843240	0.3406160	ns
PI-Cer(d18:1/14:1)	value	NL.Out	NO.In	5	5	-1.0690	0.2850494	0.3406160	ns
PE(36:6)	value	NO.In	NO.Out	5	5	1.0447	0.2961719	0.3536082	ns
LPE(18:2)	value	NL.Out	NO.In	5	5	1.0427	0.2970822	0.3543963	ns
DGDG(30:0)	value	NL.Out	NO.Out	5	5	1.0164	0.3094593	0.3643770	ns
DGTS(30:3)	value	NL.In	NO.In	5	5	1.0214	0.3070798	0.3643770	ns
LPC(18:2)	value	NL.In	NL.Out	5	5	-1.0160	0.3096414	0.3643770	ns
MGDG(34:5)	value	NL.Out	NO.In	5	5	1.0160	0.3096414	0.3643770	ns
MGTS(15:0)	value	NO.In	NO.Out	5	5	-1.0171	0.3090947	0.3643770	ns
PC(34:1)	value	NL.In	NL.Out	5	5	-1.0156	0.3098234	0.3643770	ns
PC(34:2)	value	NL.In	NO.In	5	5	-1.0156	0.3098234	0.3643770	ns
PC(34:3)	value	NL.Out	NO.In	5	5	-1.0156	0.3098234	0.3643770	ns
PC(37:7)	value	NL.Out	NO.Out	5	5	-1.0190	0.3081809	0.3643770	ns
PC(38:1)	value	NL.In	NO.In	5	5	-1.0179	0.3087296	0.3643770	ns
PE(32:2)	value	NL.In	NO.In	5	5	-1.0160	0.3096414	0.3643770	ns
PG(34:1)	value	NL.Out	NO.Out	5	5	-1.0156	0.3098234	0.3643770	ns
PG(36:2)	value	NL.In	NO.In	5	5	-1.0164	0.3094593	0.3643770	ns
PG(36:5)	value	NL.Out	NO.In	5	5	1.0156	0.3098234	0.3643770	ns
PI(32:1)	value	NL.Out	NO.In	5	5	1.0156	0.3098234	0.3643770	ns
PI(40:10)	value	NO.In	NO.Out	5	5	1.0214	0.3070798	0.3643770	ns
SQDG(34:2)	value	NL.In	NL.Out	5	5	-1.0171	0.3090947	0.3643770	ns
DGMG(20:5)	value	NL.In	NO.In	5	5	-0.9991	0.3177659	0.3734079	ns
PC(31:1)	value	NL.In	NO.In	5	5	-0.9676	0.3332380	0.3912645	ns
DGTS(33:1)	value	NL.In	NL.Out	5	5	0.9654	0.3343383	0.3922311	ns
DGTS(38:8)	value	NL.In	NO.In	5	5	-0.9621	0.3359790	0.3930479	ns
LPC(20:4)	value	NL.In	NL.Out	5	5	0.9625	0.3357973	0.3930479	ns
MGTS(18:2)	value	NL.Out	NO.Out	5	5	-0.9621	0.3359790	0.3930479	ns
PE(32:3)	value	NL.Out	NO.Out	5	5	-0.9618	0.3361448	0.3930479	ns
PE(36:8)	value	NL.In	NO.Out	5	5	0.9517	0.3412734	0.3987154	ns
PE(36:7)	value	NO.In	NO.Out	5	5	0.9509	0.3416488	0.3988250	ns
DGTS(37:5)	value	NL.Out	NO.In	5	5	-0.9386	0.3479385	0.4051652	ns
PE(30:3)	value	NL.Out	NO.In	5	5	-0.9393	0.3475733	0.4051652	ns
PE(34:3)	value	NL.In	NO.In	5	5	0.9386	0.3479385	0.4051652	ns
DGTS(38:9)	value	NL.In	NL.Out	5	5	0.9361	0.3492122	0.4063143	ns
LPE(16:2)	value	NL.In	NL.Out	5	5	0.9177	0.3587746	0.4170976	ns
PE(38:9)	value	NL.In	NL.Out	5	5	0.9156	0.3598775	0.4176939	ns
PI(30:1)	value	NL.Out	NO.In	5	5	-0.9156	0.3598775	0.4176939	ns
PE(34:6)	value	NL.In	NO.Out	5	5	0.9138	0.3608342	0.4184613	ns
DGDG(32:5)	value	NL.In	NO.In	5	5	0.9114	0.3620670	0.4188258	ns
DGDG(36:7)	value	NL.In	NL.Out	5	5	-0.9087	0.3635147	0.4188258	ns
DGTS(34:6)	value	NL.In	NO.In	5	5	0.9107	0.3624298	0.4188258	ns

DGTS(38:5)	value	NL.In	NO.In	5	5	-0.9094	0.3631537	0.4188258	ns
LPE(16:1)	value	NL.In	NO.Out	5	5	0.9087	0.3635147	0.4188258	ns
LPE(20:5)	value	NL.In	NO.In	5	5	0.9087	0.3635147	0.4188258	ns
PC(35:1)	value	NL.Out	NO.In	5	5	0.9111	0.3622485	0.4188258	ns
PG(36:5(OH))	value	NL.In	NO.Out	5	5	0.9090	0.3633343	0.4188258	ns
LPC(22:5)	value	NL.In	NO.In	5	5	0.9071	0.3643317	0.4194258	ns
MGTS(14:1)	value	NL.Out	NO.Out	5	5	-0.8959	0.3703175	0.4259704	ns
PC(31:2)	value	NL.In	NO.In	5	5	-0.8945	0.3710587	0.4264765	ns
PC(37:4)	value	NL.Out	NO.Out	5	5	0.8846	0.3763580	0.4318663	ns
SQDG(28:0)	value	NL.In	NO.Out	5	5	-0.8846	0.3763580	0.4318663	ns
LPC(17:1)	value	NL.In	NO.In	5	5	0.8634	0.3879236	0.4447772	ns
PC(37:2)	value	NL.Out	NO.In	5	5	0.8585	0.3906333	0.4475217	ns
LPE(18:4)	value	NL.In	NO.Out	5	5	0.8568	0.3915288	0.4480735	ns
MGTS(18:5)	value	NL.Out	NO.Out	5	5	0.8559	0.3920643	0.4480735	ns
PG(35:5)	value	NL.Out	NO.Out	5	5	0.8559	0.3920643	0.4480735	ns
DGTS(36:4)	value	NL.In	NL.Out	5	5	-0.8552	0.3924205	0.4481189	ns
SQDG(32:3)	value	NL.In	NO.Out	5	5	-0.8377	0.4022032	0.4589200	ns
DGDG(32:5)	value	NL.In	NL.Out	5	5	0.8310	0.4059663	0.4622987	ns
PC(37:4)	value	NL.In	NL.Out	5	5	0.8310	0.4059663	0.4622987	ns
PC(37:6)	value	NL.Out	NO.In	5	5	-0.8307	0.4061438	0.4622987	ns
SQDG(32:2)	value	NO.In	NO.Out	5	5	-0.8288	0.4072054	0.4631348	ns
DGDG(32:3)	value	NL.In	NO.In	5	5	-0.8051	0.4207504	0.4750096	ns
DGTS(32:1)	value	NL.In	NL.Out	5	5	-0.8018	0.4226781	0.4750096	ns
DGTS(33:2)	value	NL.In	NL.Out	5	5	-0.8027	0.4221542	0.4750096	ns
LPE(18:3)	value	NL.In	NO.Out	5	5	-0.8039	0.4214535	0.4750096	ns
LPE(20:3)	value	NL.In	NO.In	5	5	-0.8021	0.4225036	0.4750096	ns
MGTS(14:0)	value	NL.Out	NO.Out	5	5	-0.8018	0.4226781	0.4750096	ns
MGTS(16:2)	value	NO.In	NO.Out	5	5	-0.8021	0.4225036	0.4750096	ns
MGTS(20:4)	value	NO.In	NO.Out	5	5	-0.8018	0.4226781	0.4750096	ns
PC(35:4)	value	NO.In	NO.Out	5	5	0.8027	0.4221542	0.4750096	ns
PC(38:3)	value	NL.In	NO.In	5	5	-0.8091	0.4184477	0.4750096	ns
PC(38:6)	value	NL.In	NO.In	5	5	-0.8018	0.4226781	0.4750096	ns
PC(38:8)	value	NL.Out	NO.Out	5	5	-0.8018	0.4226781	0.4750096	ns
PI(34:3)	value	NL.Out	NO.In	5	5	-0.8082	0.4189815	0.4750096	ns
PI(36:6)	value	NL.Out	NO.In	5	5	-0.8085	0.4188037	0.4750096	ns
SQDG(34:1)	value	NL.In	NO.Out	5	5	0.8027	0.4221542	0.4750096	ns
PE(36:7)	value	NL.In	NO.Out	5	5	-0.7879	0.4307558	0.4837036	ns
SQDG(32:3)	value	NL.In	NL.Out	5	5	-0.7836	0.4332466	0.4861150	ns
PC(32:5)	value	NL.Out	NO.Out	5	5	-0.7628	0.4455783	0.4995557	ns
PE(32:4)	value	NO.In	NO.Out	5	5	0.7613	0.4464680	0.5001572	ns
PC(34:7)	value	NL.Out	NO.Out	5	5	-0.7601	0.4471711	0.5005489	ns
PC(38:3)	value	NL.Out	NO.Out	5	5	-0.7552	0.4501433	0.5034778	ns
MGTS(17:2)	value	NL.In	NO.Out	5	5	0.7523	0.4518698	0.5050100	ns
LPC(18:3)	value	NO.In	NO.Out	5	5	0.7489	0.4539206	0.5060838	ns
PC(32:4)	value	NL.Out	NO.Out	5	5	0.7497	0.4534101	0.5060838	ns
PE(40:8)	value	NL.In	NO.In	5	5	-0.7483	0.4542602	0.5060838	ns
SQDG(36:5)	value	NO.In	NO.Out	5	5	-0.7495	0.4535804	0.5060838	ns
LPC(17:2)	value	NL.In	NO.In	5	5	-0.7473	0.4548620	0.5063558	ns
PE(36:3)	value	NL.In	NO.In	5	5	-0.7162	0.4738692	0.5271004	ns
DGDG(32:2)	value	NL.In	NO.In	5	5	-0.6949	0.4871310	0.5372099	ns
DGDG(35:5)	value	NL.In	NO.In	5	5	-0.6949	0.4871310	0.5372099	ns

DGTS(38:7)	value	NL.In	NO.In	5	5	0.6951	0.4869672	0.5372099	ns
Cer(d32:1)	value	NL.In	NL.Out	5	5	-0.6951	0.4869672	0.5372099	ns
MGDG(40:8)	value	NL.Out	NO.In	5	5	0.6975	0.4854859	0.5372099	ns
MGTS(16:3)	value	NL.Out	NO.Out	5	5	-0.6949	0.4871310	0.5372099	ns
MGTS(18:3)	value	NL.Out	NO.In	5	5	-0.6951	0.4869672	0.5372099	ns
PC(32:2)	value	NL.In	NL.Out	5	5	0.6949	0.4871310	0.5372099	ns
PC(38:8)	value	NL.In	NO.In	5	5	-0.6949	0.4871310	0.5372099	ns
PE(32:1)	value	NO.In	NO.Out	5	5	-0.6970	0.4858162	0.5372099	ns
PI(33:1)	value	NL.In	NO.Out	5	5	-0.6959	0.4864748	0.5372099	ns
LPC(17:1)	value	NO.In	NO.Out	5	5	-0.6745	0.4999791	0.5509497	ns
SQDG(32:2)	value	NL.In	NL.Out	5	5	-0.6684	0.5038755	0.5548116	ns
LPC(18:5)	value	NL.In	NO.Out	5	5	0.6533	0.5135470	0.5650214	ns
PC(30:3)	value	NL.Out	NO.Out	5	5	-0.6505	0.5153437	0.5665580	ns
DGDG(36:5)	value	NL.In	NL.Out	5	5	-0.6414	0.5212453	0.5686312	ns
LPE(18:2)	value	NL.In	NO.In	5	5	-0.6417	0.5210886	0.5686312	ns
PC(30:1)	value	NL.In	NL.Out	5	5	0.6422	0.5207747	0.5686312	ns
PC(31:1)	value	NL.Out	NO.Out	5	5	-0.6451	0.5188786	0.5686312	ns
PC(34:4)	value	NL.Out	NO.Out	5	5	0.6414	0.5212453	0.5686312	ns
PC(36:4)	value	NL.In	NL.Out	5	5	-0.6414	0.5212453	0.5686312	ns
PC(37:5)	value	NO.In	NO.Out	5	5	0.6417	0.5210886	0.5686312	ns
PC(38:7)	value	NL.In	NO.Out	5	5	-0.6417	0.5210886	0.5686312	ns
PE(32:4)	value	NL.In	NO.Out	5	5	0.6442	0.5194516	0.5686312	ns
PG(31:0)	value	NL.Out	NO.Out	5	5	0.6463	0.5180820	0.5686312	ns
DGMG(16:1)	value	NL.In	NO.Out	5	5	-0.6156	0.5381405	0.5855918	ns
DGTS(36:7)	value	NL.Out	NO.In	5	5	0.6152	0.5384467	0.5855918	ns
PC(35:1)	value	NL.In	NL.Out	5	5	-0.6163	0.5376801	0.5855918	ns
PG(35:5)	value	NL.In	NO.Out	5	5	-0.6152	0.5384467	0.5855918	ns
LPC(16:0)	value	NL.In	NO.Out	5	5	-0.5880	0.5565493	0.6025029	ns
MGDG(32:2)	value	NL.Out	NO.In	5	5	0.5891	0.5558055	0.6025029	ns
PC(38:7)	value	NL.In	NL.Out	5	5	0.5882	0.5564008	0.6025029	ns
PG(30:1)	value	NL.Out	NO.Out	5	5	-0.5882	0.5564008	0.6025029	ns
PI-Cer(d18:1/14:1)	value	NL.In	NO.In	5	5	0.5880	0.5565493	0.6025029	ns
SQDG(28:0)	value	NL.In	NL.Out	5	5	0.5898	0.5553575	0.6025029	ns
MGTS(14:1)	value	NO.In	NO.Out	5	5	-0.5701	0.5686067	0.6150856	ns
SQDG(34:1)	value	NL.In	NL.Out	5	5	0.5619	0.5741960	0.6206576	ns
DGDG(38:6)	value	NL.In	NL.Out	5	5	-0.5351	0.5925625	0.6375549	ns
DGTS(32:5)	value	NL.In	NO.In	5	5	0.5380	0.5905966	0.6375549	ns
DGTS(34:2)	value	NL.In	NO.Out	5	5	-0.5347	0.5928410	0.6375549	ns
LPC(20:5)	value	NO.In	NO.Out	5	5	-0.5347	0.5928410	0.6375549	ns
LPE(14:0)	value	NL.In	NO.In	5	5	-0.5350	0.5926216	0.6375549	ns
MGTS(20:5)	value	NL.In	NO.In	5	5	0.5345	0.5929801	0.6375549	ns
PE(32:1)	value	NL.In	NO.In	5	5	-0.5361	0.5918636	0.6375549	ns
MGDG(34:1)	value	NL.In	NO.In	5	5	0.5140	0.6072452	0.6523970	ns
PC(33:2)	value	NL.In	NO.In	5	5	-0.5080	0.6114622	0.6559322	ns
PE(36:5)	value	NL.In	NO.Out	5	5	-0.5086	0.6110595	0.6559322	ns
LPC(17:2)	value	NL.In	NO.Out	5	5	-0.4982	0.6183271	0.6627942	ns
DGTS(34:5)	value	NL.In	NO.In	5	5	-0.4811	0.6304666	0.6702257	ns
DGTS(38:5)	value	NL.Out	NO.Out	5	5	-0.4814	0.6302092	0.6702257	ns
DGTS(40:9)	value	NL.In	NO.In	5	5	-0.4811	0.6304666	0.6702257	ns
LPC(20:0)	value	NL.Out	NO.Out	5	5	-0.4855	0.6273427	0.6702257	ns

MGDG(36:6)	value	NL.Out	NO.In	5	5	-0.4814	0.6302092	0.6702257	ns
PC(33:3)	value	NL.In	NL.Out	5	5	0.4816	0.6300804	0.6702257	ns
PC(36:7)	value	NL.Out	NO.Out	5	5	-0.4814	0.6302092	0.6702257	ns
PE(40:7)	value	NL.In	NL.Out	5	5	0.4813	0.6303380	0.6702257	ns
PE(40:7)	value	NL.In	NO.In	5	5	0.4813	0.6303380	0.6702257	ns
PI(40:10)	value	NL.In	NL.Out	5	5	0.4838	0.6285234	0.6702257	ns
SQDG(32:1)	value	NO.In	NO.Out	5	5	-0.4811	0.6304666	0.6702257	ns
LPC(22:5)	value	NL.In	NO.Out	5	5	0.4536	0.6501376	0.6901011	ns
LPC(22:5)	value	NO.In	NO.Out	5	5	-0.4536	0.6501376	0.6901011	ns
PC(40:11)	value	NL.In	NO.Out	5	5	0.4027	0.6871596	0.7288524	ns
PG(36:6)	value	NL.Out	NO.Out	5	5	0.3998	0.6893146	0.7305909	ns
DGDG(38:7)	value	NL.In	NO.Out	5	5	0.3744	0.7080715	0.7467803	ns
DGTS(34:3)	value	NO.In	NO.Out	5	5	-0.3743	0.7081763	0.7467803	ns
DGTS(34:4)	value	NL.In	NL.Out	5	5	0.3743	0.7081763	0.7467803	ns
PC(35:4)	value	NL.In	NL.Out	5	5	-0.3746	0.7079666	0.7467803	ns
PC(38:6)	value	NL.Out	NO.Out	5	5	0.3742	0.7082810	0.7467803	ns
PC(40:10)	value	NL.In	NO.Out	5	5	-0.3743	0.7081763	0.7467803	ns
PI(33:1)	value	NL.Out	NO.In	5	5	-0.3747	0.7078615	0.7467803	ns
LPE(16:3)	value	NL.In	NO.Out	5	5	0.3603	0.7186501	0.7571492	ns
PE(34:4)	value	NL.In	NO.Out	5	5	0.3497	0.7265777	0.7649323	ns
PC(37:6)	value	NL.In	NO.In	5	5	0.3484	0.7275717	0.7654097	ns
MGTS(14:1)	value	NL.Out	NO.In	5	5	-0.3258	0.7445953	0.7827371	ns
PE(38:9)	value	NO.In	NO.Out	5	5	0.3232	0.7465798	0.7842411	ns
LPE(20:3)	value	NL.Out	NO.Out	5	5	0.3208	0.7483361	0.7849214	ns
PE(40:10)	value	NL.In	NL.Out	5	5	-0.3208	0.7483361	0.7849214	ns
MGTS(16:4)	value	NO.In	NO.Out	5	5	0.3096	0.7568413	0.7932549	ns
PC(35:1)	value	NL.In	NO.In	5	5	0.2948	0.7681741	0.8045374	ns
PC(28:1)	value	NL.In	NO.In	5	5	-0.2837	0.7766095	0.8127709	ns
DGTS(36:8)	value	NL.Out	NO.Out	5	5	-0.2673	0.7892680	0.8223720	ns
DGTS(40:10)	value	NL.Out	NO.Out	5	5	-0.2673	0.7892680	0.8223720	ns
MGTS(17:1)	value	NL.Out	NO.Out	5	5	0.2683	0.7884902	0.8223720	ns
PC(36:3)	value	NL.In	NO.In	5	5	-0.2673	0.7892680	0.8223720	ns
PE(30:1)	value	NL.In	NO.In	5	5	0.2698	0.7873071	0.8223720	ns
PE(36:4)	value	NL.Out	NO.Out	5	5	0.2677	0.7889579	0.8223720	ns
LPC(17:2)	value	NO.In	NO.Out	5	5	0.2491	0.8032747	0.8363507	ns
DGTS(38:10)	value	NL.In	NL.Out	5	5	0.2422	0.8086411	0.8409244	ns
PG(33:1)	value	NL.In	NO.Out	5	5	0.2419	0.8088552	0.8409244	ns
SQDG(34:1)	value	NL.Out	NO.Out	5	5	0.2408	0.8097048	0.8411900	ns
DGDG(30:1)	value	NL.Out	NO.In	5	5	-0.2140	0.8305705	0.8604722	ns
DGDG(34:5)	value	NL.In	NO.Out	5	5	0.2138	0.8306960	0.8604722	ns
PC(36:4(OH))	value	NL.Out	NO.Out	5	5	-0.2144	0.8302555	0.8604722	ns
PE(38:8)	value	NL.In	NO.Out	5	5	-0.2145	0.8301290	0.8604722	ns
DGMG(20:5)	value	NL.In	NO.Out	5	5	0.1890	0.8500840	0.8787353	ns
LPC(17:1)	value	NL.In	NO.Out	5	5	0.1889	0.8501976	0.8787353	ns
PE(40:1)	value	NO.In	NO.Out	5	5	-0.1879	0.8509853	0.8787353	ns
LPG(16:0)	value	NL.In	NL.Out	5	5	-0.1873	0.8514300	0.8787353	ns
PG(31:1)	value	NL.Out	NO.Out	5	5	-0.1886	0.8504239	0.8787353	ns
PC(28:1)	value	NL.Out	NO.Out	5	5	-0.1702	0.8648182	0.8919028	ns
DGTS(38:6)	value	NL.Out	NO.Out	5	5	-0.1604	0.8726001	0.8927758	ns
DGTS(38:7)	value	NL.Out	NO.Out	5	5	-0.1604	0.8725525	0.8927758	ns
LPE(20:4)	value	NL.In	NL.Out	5	5	0.1604	0.8726001	0.8927758	ns

MGTS(20:4)	value	NL.In	NL.Out	5	5	-0.1604	0.8726001	0.8927758	ns
PC(32:1)	value	NL.Out	NO.Out	5	5	0.1604	0.8726001	0.8927758	ns
PG(32:1)	value	NL.In	NL.Out	5	5	0.1604	0.8725525	0.8927758	ns
PG(36:5)	value	NL.In	NO.In	5	5	-0.1604	0.8726001	0.8927758	ns
PI(32:2)	value	NL.In	NL.Out	5	5	0.1604	0.8726001	0.8927758	ns
PI(34:1)	value	NL.Out	NO.In	5	5	-0.1604	0.8726001	0.8927758	ns
PI(34:2)	value	NL.Out	NO.Out	5	5	0.1604	0.8726001	0.8927758	ns
SQDG(30:1)	value	NL.Out	NO.In	5	5	0.1604	0.8725525	0.8927758	ns
PE(32:4)	value	NL.In	NO.In	5	5	-0.1171	0.9067605	0.9270562	ns
DGDG(32:3)	value	NL.Out	NO.Out	5	5	0.1073	0.9145118	0.9319773	ns
DGTS(36:5)	value	NL.In	NO.In	5	5	-0.1069	0.9148647	0.9319773	ns
PC(35:3)	value	NL.Out	NO.Out	5	5	-0.1069	0.9148328	0.9319773	ns
PC(38:2)	value	NL.Out	NO.Out	5	5	0.1072	0.9146085	0.9319773	ns
PC(38:5)	value	NL.Out	NO.Out	5	5	0.1069	0.9148328	0.9319773	ns
DGDG(32:5)	value	NL.Out	NO.In	5	5	0.0804	0.9359027	0.9527234	ns
DGDG(32:2)	value	NL.Out	NO.Out	5	5	0.0535	0.9573716	0.9676218	ns
DGMG(16:0)	value	NO.In	NO.Out	5	5	0.0536	0.9572429	0.9676218	ns
DGTS(36:6)	value	NL.In	NO.In	5	5	-0.0535	0.9573556	0.9676218	ns
PC(34:2)	value	NL.Out	NO.Out	5	5	0.0535	0.9573716	0.9676218	ns
PC(35:3)	value	NL.In	NO.In	5	5	0.0535	0.9573556	0.9676218	ns
PC(36:3)	value	NL.Out	NO.Out	5	5	-0.0535	0.9573716	0.9676218	ns
PE(38:7)	value	NL.Out	NO.Out	5	5	-0.0536	0.9572752	0.9676218	ns
PE(40:9)	value	NL.In	NO.Out	5	5	-0.0535	0.9573716	0.9676218	ns
PI(34:5)	value	NL.In	NL.Out	5	5	-0.0537	0.9571781	0.9676218	ns
SQDG(32:3)	value	NL.Out	NO.Out	5	5	-0.0540	0.9568995	0.9676218	ns
DGMG(14:0)	value	NL.In	NO.Out	5	5	0.0000	1.0000000	1.0000000	ns
DGTS(36:3)	value	NL.In	NL.Out	5	5	0.0000	1.0000000	1.0000000	ns
DGTS(36:3)	value	NL.In	NO.In	5	5	0.0000	1.0000000	1.0000000	ns
DGTS(36:3)	value	NL.Out	NO.In	5	5	0.0000	1.0000000	1.0000000	ns
LPC(20:1)	value	NL.In	NO.Out	5	5	0.0000	1.0000000	1.0000000	ns
LPE(20:2)	value	NL.In	NL.Out	5	5	0.0000	1.0000000	1.0000000	ns
MGDG(34:1)	value	NL.Out	NO.Out	5	5	0.0000	1.0000000	1.0000000	ns
MGDG(34:2)	value	NL.In	NO.In	5	5	0.0000	1.0000000	1.0000000	ns
MGDG(34:2)	value	NL.Out	NO.Out	5	5	0.0000	1.0000000	1.0000000	ns
PE(40:7)	value	NL.Out	NO.In	5	5	0.0000	1.0000000	1.0000000	ns
PG(32:2)	value	NL.Out	NO.Out	5	5	0.0000	1.0000000	1.0000000	ns
PG(36:6)	value	NL.In	NO.In	5	5	0.0000	1.0000000	1.0000000	ns
PI(28:0)	value	NL.In	NL.Out	5	5	0.0000	1.0000000	1.0000000	ns
PI(28:0)	value	NL.In	NO.In	5	5	0.0000	1.0000000	1.0000000	ns
PI(28:0)	value	NL.Out	NO.In	5	5	0.0000	1.0000000	1.0000000	ns

Table S5.14. Polar lipids showing significant differences (*p* value < 0.05) and up- or downregulation in the pairwise lipid expression profiling analysis (Welch's t-test). Comparison between *N. oceanica* (NO) grown indoors (In) and NO grown outdoors (Out). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

Label	*p*	NO-In	NO-Out	log2FC	Regulation
MGDG(36:5)	0.0000	5.4680	0.0660	6.3724	UP in the NO-In group
MGDG(38:7)	0.0000	0.4600	0.0060	6.2605	UP in the NO-In group
MGDG(40:8)	0.0000	0.8200	0.0120	6.0945	UP in the NO-In group
MGDG(40:9)	0.0003	1.2360	0.0340	5.1840	UP in the NO-In group
MGDG(32:2)	0.0009	0.4880	0.0144	5.0827	UP in the NO-In group
MGDG(32:1)	0.0002	5.6200	0.1880	4.9018	UP in the NO-In group
MGDG(34:5)	0.0000	5.2940	0.2800	4.2409	UP in the NO-In group
MGDG(30:1)	0.0000	0.4500	0.0240	4.2288	UP in the NO-In group
MGDG(34:1)	0.0000	0.3960	0.0240	4.0444	UP in the NO-In group
PC(36:2)	0.0000	18.2860	1.1700	3.9662	UP in the NO-In group
MGDG(36:6)	0.0000	1.0920	0.0960	3.5078	UP in the NO-In group
MGDG(34:2)	0.0000	0.5680	0.0520	3.4493	UP in the NO-In group
DGDG(34:1)	0.0000	1.6960	0.1600	3.4060	UP in the NO-In group
DGDG(34:2)	0.0000	3.3140	0.3180	3.3815	UP in the NO-In group
PC(37:2)	0.0000	0.4660	0.0460	3.3406	UP in the NO-In group
PE(38:5)	0.0000	2.8580	0.2940	3.2811	UP in the NO-In group
DGTS(36:2)	0.0000	0.2720	0.0280	3.2801	UP in the NO-In group
LPC(18:1)	0.0000	12.1760	1.3280	3.1967	UP in the NO-In group
PC(38:1)	0.0000	0.4120	0.0500	3.0426	UP in the NO-In group
LPE(18:1)	0.0000	1.9140	0.2480	2.9482	UP in the NO-In group
DGTS(34:1)	0.0000	1.8800	0.2600	2.8541	UP in the NO-In group
PE(36:3)	0.0000	0.0280	0.0040	2.8074	UP in the NO-In group
MGDG(32:5)	0.0000	0.5100	0.0740	2.7849	UP in the NO-In group
MGTS(17:2)	0.0000	0.6720	0.1040	2.6919	UP in the NO-In group
LPC(20:1)	0.0034	0.0520	0.0084	2.6301	UP in the NO-In group
PC(34:1)	0.0000	31.3140	5.2280	2.5825	UP in the NO-In group
PC(38:3)	0.0001	0.1640	0.0280	2.5502	UP in the NO-In group
MGDG(40:10)	0.0000	2.6440	0.4680	2.4981	UP in the NO-In group
MGTS(18:1)	0.0000	3.3100	0.6120	2.4352	UP in the NO-In group
PC(38:5)	0.0000	12.4880	2.4100	2.3734	UP in the NO-In group
DGTS(33:1)	0.0000	0.8540	0.1660	2.3631	UP in the NO-In group
DGTS(34:2)	0.0000	7.4320	1.4620	2.3458	UP in the NO-In group
PE(38:6)	0.0000	2.9620	0.6000	2.3035	UP in the NO-In group
MGTS(17:1)	0.0000	0.5200	0.1080	2.2675	UP in the NO-In group
MGTS(18:2)	0.0000	6.2500	1.3520	2.2088	UP in the NO-In group
DGDG(32:1)	0.0000	23.3080	5.1880	2.1676	UP in the NO-In group
DGTS(33:2)	0.0000	1.2140	0.2880	2.0756	UP in the NO-In group
LPE(18:2)	0.0000	1.0280	0.2500	2.0398	UP in the NO-In group
PC(36:4(OH))	0.0000	0.7760	0.1980	1.9706	UP in the NO-In group
PC(37:3)	0.0000	0.2240	0.0600	1.9005	UP in the NO-In group
Cer(d32:1)	0.0000	3.9520	1.0780	1.8742	UP in the NO-In group
PC(35:2)	0.0000	1.5500	0.4620	1.7463	UP in the NO-In group
PC(36:3)	0.0000	11.4140	3.4120	1.7421	UP in the NO-In group
DGDG(34:3)	0.0000	1.1940	0.3720	1.6824	UP in the NO-In group
DGTS(36:4)	0.0000	14.8200	4.7280	1.6482	UP in the NO-In group
DGTS(38:5)	0.0000	1.1620	0.3840	1.5974	UP in the NO-In group
SQDG(34:1)	0.0001	2.3320	0.7780	1.5837	UP in the NO-In group
MGTS(16:0)	0.0000	6.4080	2.2780	1.4921	UP in the NO-In group
DGDG(30:0)	0.0000	0.5000	0.1800	1.4739	UP in the NO-In group

SQDG(34:2)	0.0000	1.3660	0.4940	1.4674	UP in the NO-In group
DGDG(32:3)	0.0000	0.3080	0.1120	1.4594	UP in the NO-In group
PC(38:2)	0.0000	0.3080	0.1120	1.4594	UP in the NO-In group
PC(35:1)	0.0000	0.2920	0.1080	1.4349	UP in the NO-In group
PI-Cer(d18:1/14:0)	0.0006	4.9120	1.9060	1.3658	UP in the NO-In group
DGDG(35:5)	0.0000	1.6080	0.6320	1.3473	UP in the NO-In group
PC(40:8)	0.0000	4.1600	1.7000	1.2910	UP in the NO-In group
DGDG(38:7)	0.0000	1.2720	0.5240	1.2795	UP in the NO-In group
PE(30:0)	0.0343	0.0200	0.0084	1.2515	UP in the NO-In group
DGDG(36:7)	0.0000	3.1220	1.4520	1.1044	UP in the NO-In group
MGMG(16:0)	0.0003	0.3440	0.1600	1.1043	UP in the NO-In group
DGTS(38:6)	0.0003	2.5020	1.1860	1.0770	UP in the NO-In group
PG(34:1)	0.0000	1.5640	0.7460	1.0680	UP in the NO-In group
PI(34:1)	0.0002	9.3700	4.4860	1.0626	UP in the NO-In group
MGTS(18:3)	0.0001	3.9700	1.9200	1.0480	UP in the NO-In group
PC(35:3)	0.0000	1.2940	0.6280	1.0430	UP in the NO-In group
PC(40:5)	0.0001	0.1260	0.0620	1.0231	UP in the NO-In group
SQDG(32:3)	0.0178	0.1420	0.0700	1.0205	UP in the NO-In group
LPE(20:5)	0.0003	6.4940	3.2700	0.9898	-
PE(38:7)	0.0000	0.8680	0.4420	0.9736	-
DGTS(38:7)	0.0008	4.0260	2.0620	0.9653	-
PG(36:5)	0.0005	86.8480	44.5200	0.9640	-
LPC(20:4)	0.0001	3.5440	1.8600	0.9301	-
LPC(18:2)	0.0003	3.6080	1.9180	0.9116	-
DGDG(32:2)	0.0001	5.0240	2.6800	0.9066	-
PE(40:10)	0.0001	0.5120	0.2740	0.9020	-
PI(34:5)	0.0055	1.0140	0.5440	0.8984	-
PC(34:2)	0.0000	34.4980	18.5960	0.8915	-
LPE(16:0)	0.0003	0.0600	0.0340	0.8194	-
DGDG(36:5)	0.0001	25.8660	15.2700	0.7604	-
PI(32:1)	0.0021	48.2460	30.0320	0.6839	-
PG(36:2)	0.0134	0.4880	0.3040	0.6828	-
LPG(16:0)	0.0098	0.6260	0.3920	0.6753	-
DGTS(36:5)	0.0068	20.1100	13.2860	0.5980	-
PC(36:4)	0.0016	11.7340	7.7600	0.5966	-
PG(35:5)	0.0125	1.0820	0.7160	0.5957	-
PC(38:6)	0.0005	7.3000	4.8560	0.5881	-
PI(33:1)	0.0145	0.7620	0.5200	0.5513	-
MGTS(18:5)	0.0045	0.7020	0.4920	0.5128	-
DGTS(38:8)	0.0388	3.6040	2.6400	0.4491	-
DGTS(32:1)	0.0178	23.8420	17.7560	0.4252	-
PE(40:9)	0.0088	2.2740	1.7400	0.3861	-
MGTS(15:0)	0.0396	0.2520	0.1960	0.3626	-
DGMG(16:1)	0.0091	0.2300	0.1860	0.3063	-
DGDG(38:6)	0.0198	0.4420	0.3580	0.3041	-
DGTS(37:5)	0.1240	0.4120	0.3380	0.2856	-
PG(34:2)	0.1640	1.2760	1.0560	0.2730	-
MGTS(16:2)	0.0718	1.7000	1.4100	0.2698	-
SQDG(31:1)	0.0165	1.9900	1.6520	0.2686	-
SQDG(36:5)	0.2000	0.1940	0.1620	0.2601	-

LPC(22:5)	0.3740	0.0100	0.0084	0.2515	-
MGTS(20:4)	0.1300	24.1500	20.5440	0.2333	-
LPC(20:5)	0.2140	2.1320	1.8440	0.2094	-
SQDG(32:2)	0.0845	4.1380	3.5860	0.2066	-
PC(37:4)	0.2330	0.3360	0.2960	0.1829	-
SQDG(30:0)	0.1420	13.0000	11.5580	0.1696	-
MGTS(14:1)	0.3880	0.0720	0.0660	0.1255	-
SQDG(32:1)	0.3310	130.8640	120.2520	0.1220	-
LPC(17:1)	0.4310	0.1280	0.1180	0.1174	-
PE(40:1)	0.5700	0.1900	0.1760	0.1104	-
DGTS(34:3)	0.4750	3.2800	3.0480	0.1058	-
PE(32:1)	0.4100	0.2740	0.2580	0.0868	-
PE(30:3)	0.8380	0.6760	0.6640	0.0258	-
DGMG(16:0)	0.9940	0.3080	0.3080	0.0000	-
LPC(17:2)	0.8050	0.0640	0.0660	-0.0444	-
PE(38:9)	0.5160	0.0840	0.0880	-0.0671	-
MGTS(16:4)	0.3740	0.0300	0.0320	-0.0931	-
PC(35:4)	0.3760	0.5080	0.5520	-0.1198	-
LPC(18:3)	0.4950	0.4520	0.4920	-0.1223	-
PC(40:9)	0.1750	2.4620	2.7280	-0.1480	-
PC(37:5)	0.2180	0.7940	0.8940	-0.1711	-
PE(36:7)	0.1480	0.0700	0.0800	-0.1926	-
LPC(16:0)	0.1730	1.2300	1.4200	-0.2072	-
PC(30:3)	0.1700	0.5760	0.6900	-0.2605	-
PE(32:4)	0.1780	0.0200	0.0240	-0.2630	-
PE(36:6)	0.0785	0.3080	0.3700	-0.2646	-
DGTS(34:4)	0.0470	8.6520	10.6260	-0.2965	-
PC(38:7)	0.0141	2.5520	3.1380	-0.2982	-
DGDG(30:1)	0.0159	1.1060	1.3740	-0.3130	-
MGTS(18:4)	0.0348	5.6340	7.1080	-0.3353	-
LPE(20:4)	0.0248	11.6660	15.2160	-0.3833	-
LPE(20:2)	0.0544	0.0640	0.0860	-0.4263	-
PC(36:5)	0.0014	12.0820	16.5240	-0.4517	-
MGTS(16:1)	0.0140	17.4260	23.9280	-0.4575	-
PG(32:1)	0.0098	7.1600	10.1340	-0.5012	-
DGMG(20:5)	0.1310	0.0480	0.0680	-0.5025	-
PE(34:3)	0.0030	0.0840	0.1200	-0.5146	-
LPE(18:3)	0.0070	0.1160	0.1660	-0.5171	-
PC(37:6)	0.0020	0.5240	0.7500	-0.5173	-
PE(36:5)	0.0017	0.5600	0.8060	-0.5254	-
PE(30:1)	0.0062	0.0540	0.0780	-0.5305	-
DGDG(34:5)	0.0016	6.9800	10.1000	-0.5331	-
PE(38:8)	0.0023	0.2100	0.3040	-0.5337	-
DGDG(36:6)	0.0011	16.1120	23.3800	-0.5371	-
PE(34:4)	0.0018	0.1200	0.1760	-0.5525	-
DGDG(32:5)	0.0064	0.2960	0.4380	-0.5653	-
MGTS(20:5)	0.0044	44.4420	66.3720	-0.5787	-
SQDG(28:0)	0.0118	0.2380	0.3580	-0.5890	-
DGTS(38:10)	0.0230	0.0660	0.1020	-0.6280	-
PC(37:7)	0.0003	0.1840	0.3020	-0.7148	-
PE(40:8)	0.0004	2.6060	4.3580	-0.7418	-

PI(40:10)	0.0364	0.3380	0.5680	-0.7489	-
PC(40:10)	0.0001	0.4840	0.8340	-0.7850	-
MGTS(16:3)	0.0009	1.3120	2.3560	-0.8446	-
DGTS(36:7)	0.0006	0.8300	1.6260	-0.9701	-
PC(28:1)	0.0038	0.0180	0.0360	-1.0000	-
DGTS(32:2)	0.0000	6.1960	12.5640	-1.0199	DOWN in the NO-In group
SQDG(30:1)	0.0001	4.2320	8.7720	-1.0516	DOWN in the NO-In group
PC(34:3)	0.0000	8.4620	17.9200	-1.0825	DOWN in the NO-In group
MGTS(14:0)	0.0006	2.6300	5.5860	-1.0868	DOWN in the NO-In group
PC(32:1)	0.0000	4.4420	9.6660	-1.1217	DOWN in the NO-In group
LPE(14:0)	0.0007	0.0100	0.0220	-1.1375	DOWN in the NO-In group
PC(40:11)	0.0000	0.0360	0.0800	-1.1520	DOWN in the NO-In group
PI(34:2)	0.0001	1.1520	2.5980	-1.1733	DOWN in the NO-In group
PE(36:8)	0.0001	0.0200	0.0460	-1.2016	DOWN in the NO-In group
PC(31:1)	0.0000	0.0860	0.2000	-1.2176	DOWN in the NO-In group
PE(36:4)	0.0000	0.1680	0.3960	-1.2370	DOWN in the NO-In group
DGTS(34:6)	0.0000	0.1560	0.3680	-1.2382	DOWN in the NO-In group
PC(38:8)	0.0000	1.9320	4.5860	-1.2471	DOWN in the NO-In group
DGTS(32:3)	0.0000	0.7040	1.7040	-1.2753	DOWN in the NO-In group
DGTS(40:9)	0.0000	6.7640	16.8660	-1.3182	DOWN in the NO-In group
LPE(16:2)	0.0010	0.0200	0.0500	-1.3219	DOWN in the NO-In group
PC(33:2)	0.0000	0.5740	1.4720	-1.3587	DOWN in the NO-In group
PE(34:2)	0.0000	0.1320	0.3400	-1.3650	DOWN in the NO-In group
PE(34:5)	0.0000	0.0800	0.2120	-1.4060	DOWN in the NO-In group
PC(34:4)	0.0000	1.8820	5.0060	-1.4114	DOWN in the NO-In group
LPE(16:1)	0.0000	0.2960	0.8180	-1.4665	DOWN in the NO-In group
LPC(18:5)	0.0004	0.0180	0.0500	-1.4739	DOWN in the NO-In group
DGTS(36:6)	0.0000	3.6540	10.2300	-1.4853	DOWN in the NO-In group
LPE(18:4)	0.0000	0.1100	0.3080	-1.4854	DOWN in the NO-In group
DGTS(30:2)	0.0000	0.4440	1.2580	-1.5025	DOWN in the NO-In group
LPC(22:6)	0.0000	0.0340	0.1000	-1.5564	DOWN in the NO-In group
PE(32:2)	0.0000	0.1880	0.5640	-1.5850	DOWN in the NO-In group
DGTS(32:5)	0.0000	0.1440	0.4400	-1.6114	DOWN in the NO-In group
LPC(16:1)	0.0000	2.0020	6.1560	-1.6206	DOWN in the NO-In group
DGTS(38:9)	0.0000	0.6420	1.9760	-1.6219	DOWN in the NO-In group
LPC(16:2)	0.0000	0.1060	0.3300	-1.6384	DOWN in the NO-In group
LPC(18:4)	0.0000	0.3740	1.1660	-1.6405	DOWN in the NO-In group
PG(33:1)	0.0004	0.0340	0.1140	-1.7454	DOWN in the NO-In group
DGTS(34:5)	0.0000	6.3840	21.9500	-1.7817	DOWN in the NO-In group
PC(33:3)	0.0000	0.1960	0.6780	-1.7904	DOWN in the NO-In group
DGTS(40:10)	0.0000	7.8660	27.4820	-1.8048	DOWN in the NO-In group
Cer(d32:2)	0.0000	0.4960	1.7440	-1.8140	DOWN in the NO-In group
LPC(20:3)	0.0000	0.1640	0.5800	-1.8224	DOWN in the NO-In group
PI(30:1)	0.0000	2.4400	8.7300	-1.8391	DOWN in the NO-In group
LPC(14:0)	0.0000	0.0540	0.1960	-1.8598	DOWN in the NO-In group
PC(36:6)	0.0000	3.7180	13.9080	-1.9033	DOWN in the NO-In group
PG(36:5(OH))	0.0002	2.0300	7.6800	-1.9196	DOWN in the NO-In group
PC(36:7)	0.0000	0.2500	0.9560	-1.9351	DOWN in the NO-In group
LPC(16:3)	0.0001	0.0120	0.0460	-1.9386	DOWN in the NO-In group
DGTS(36:8)	0.0000	0.6520	2.6460	-2.0209	DOWN in the NO-In group
DGDG(40:10)	0.0000	0.6740	2.8180	-2.0639	DOWN in the NO-In group

PC(38:10)	0.0000	0.0300	0.1280	-2.0931	DOWN in the NO-In group
PC(31:2)	0.0000	0.0220	0.0940	-2.0952	DOWN in the NO-In group
DGMG(14:0)	0.0095	0.0068	0.0300	-2.1414	DOWN in the NO-In group
PI(32:2)	0.0000	1.3580	6.0120	-2.1464	DOWN in the NO-In group
DGTS(32:4)	0.0000	1.7300	8.0800	-2.2236	DOWN in the NO-In group
PI-Cer(d18:1/14:1)	0.0000	1.0200	4.8300	-2.2435	DOWN in the NO-In group
PE(32:3)	0.0000	0.0100	0.0480	-2.2630	DOWN in the NO-In group
PC(38:9)	0.0000	0.6520	3.1820	-2.2870	DOWN in the NO-In group
PE(40:7)	0.0000	0.7480	3.8200	-2.3525	DOWN in the NO-In group
PC(30:1)	0.0000	0.2940	1.5420	-2.3909	DOWN in the NO-In group
PC(32:5)	0.0000	0.0200	0.1060	-2.4060	DOWN in the NO-In group
LPC(20:0)	0.0000	0.0200	0.1100	-2.4594	DOWN in the NO-In group
DGTS(28:1)	0.0000	0.0840	0.4660	-2.4719	DOWN in the NO-In group
DGTS(36:3)	0.0000	0.1300	0.7240	-2.4775	DOWN in the NO-In group
DGTS(30:1)	0.0000	7.6300	42.9300	-2.4922	DOWN in the NO-In group
PI(34:3)	0.0000	0.1180	0.6700	-2.5054	DOWN in the NO-In group
PI(36:6)	0.0000	0.1140	0.6500	-2.5114	DOWN in the NO-In group
PC(32:4)	0.0000	0.1220	0.7100	-2.5409	DOWN in the NO-In group
LPE(16:3)	0.0002	0.0020	0.0120	-2.5850	DOWN in the NO-In group
LPE(20:3)	0.0000	0.3520	2.1160	-2.5877	DOWN in the NO-In group
PG(34:5)	0.0000	0.3480	2.0980	-2.5919	DOWN in the NO-In group
PG(36:6)	0.0000	6.8280	44.5280	-2.7052	DOWN in the NO-In group
PI(28:0)	0.0000	0.0440	0.3000	-2.7694	DOWN in the NO-In group
PC(34:5)	0.0000	0.9760	6.7340	-2.7865	DOWN in the NO-In group
PE(36:2)	0.0000	0.0040	0.0280	-2.8074	DOWN in the NO-In group
DGTS(30:3)	0.0000	0.0620	0.4660	-2.9100	DOWN in the NO-In group
PC(36:8)	0.0000	0.0400	0.3060	-2.9355	DOWN in the NO-In group
PC(32:2)	0.0000	3.3900	26.7520	-2.9803	DOWN in the NO-In group
PE(34:6)	0.0003	0.0020	0.0160	-3.0000	DOWN in the NO-In group
LPC(17:0)	0.0000	0.0020	0.0200	-3.3219	DOWN in the NO-In group
DGTS(28:0)	0.0000	0.1740	1.8160	-3.3836	DOWN in the NO-In group
PC(32:3)	0.0000	0.3080	3.3240	-3.4319	DOWN in the NO-In group
PC(34:6)	0.0000	0.0700	0.7600	-3.4406	DOWN in the NO-In group
PG(31:0)	0.0000	0.0780	1.2200	-3.9673	DOWN in the NO-In group
PG(32:2)	0.0000	0.0860	1.7100	-4.3135	DOWN in the NO-In group
PG(30:1)	0.0000	0.0640	1.4980	-4.5488	DOWN in the NO-In group
PC(34:7)	0.0000	0.0020	0.0520	-4.7004	DOWN in the NO-In group
PG(31:1)	0.0000	0.0040	0.4060	-6.6653	DOWN in the NO-In group

Table S5.15. Polar lipids showing significant differences (*p* value < 0.05) and up- or downregulation in the pairwise lipid expression profiling analysis (Welch's t-test). Comparison between *N. limnetica* (NL) grown indoors (In) and NL grown outdoors (Out). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

Label	*p*	NL-In	NL-Out	log2FC	Regulation
MGDG(38:7)	0.0000	1.7140	0.0500	5.0993	UP in the NL-In group
PI-Cer(d18:1/14:0)	0.0015	0.1320	0.0040	5.0444	UP in the NL-In group
PC(38:2)	0.0000	2.0040	0.1100	4.1873	UP in the NL-In group
MGDG(34:1)	0.0004	0.3240	0.0240	3.7549	UP in the NL-In group
MGDG(34:2)	0.0002	0.5280	0.0520	3.3440	UP in the NL-In group
MGDG(32:1)	0.0003	10.4860	1.1800	3.1516	UP in the NL-In group
PC(38:1)	0.0000	0.6020	0.0920	2.7101	UP in the NL-In group
PC(40:5)	0.0000	0.5440	0.0860	2.6612	UP in the NL-In group
MGDG(30:1)	0.0004	1.3460	0.2460	2.4519	UP in the NL-In group
PC(38:3)	0.0000	0.1900	0.0360	2.3999	UP in the NL-In group
MGDG(40:8)	0.0000	3.5560	0.7100	2.3244	UP in the NL-In group
DGDG(34:2)	0.0002	1.9580	0.4660	2.0710	UP in the NL-In group
MGDG(32:2)	0.0000	1.6580	0.4300	1.9470	UP in the NL-In group
DGDG(32:3)	0.0002	0.3900	0.1060	1.8794	UP in the NL-In group
MGDG(34:5)	0.0000	15.5860	4.3300	1.8478	UP in the NL-In group
DGDG(34:3)	0.0000	0.7020	0.1980	1.8260	UP in the NL-In group
DGTS(38:5)	0.0001	1.4640	0.4280	1.7742	UP in the NL-In group
MGDG(36:5)	0.0000	13.3140	3.9240	1.7625	UP in the NL-In group
PC(38:5)	0.0000	8.2260	2.4660	1.7380	UP in the NL-In group
PC(36:2)	0.0001	9.3020	2.8480	1.7076	UP in the NL-In group
PC(36:3)	0.0000	11.3180	3.5760	1.6622	UP in the NL-In group
MGMG(16:0)	0.0000	0.7720	0.2440	1.6617	UP in the NL-In group
DGTS(38:6)	0.0000	3.5600	1.2220	1.5426	UP in the NL-In group
PE(40:8)	0.0005	3.1100	1.1280	1.4631	UP in the NL-In group
DGDG(34:1)	0.0003	1.1640	0.4260	1.4502	UP in the NL-In group
MGDG(32:5)	0.0000	2.2900	0.8880	1.3667	UP in the NL-In group
DGDG(32:1)	0.0011	26.6060	10.5200	1.3386	UP in the NL-In group
DGDG(30:0)	0.0008	0.3160	0.1260	1.3265	UP in the NL-In group
MGDG(36:6)	0.0004	2.8900	1.2060	1.2608	UP in the NL-In group
MGDG(40:9)	0.0001	5.4720	2.3780	1.2023	UP in the NL-In group
LPC(20:0)	0.0001	0.2760	0.1200	1.2016	UP in the NL-In group
PC(40:8)	0.0005	2.8060	1.2680	1.1460	UP in the NL-In group
PE(38:5)	0.0000	1.6640	0.7600	1.1306	UP in the NL-In group
PC(34:2)	0.0000	40.3120	18.5460	1.1201	UP in the NL-In group
DGDG(32:2)	0.0005	5.8160	2.6880	1.1135	UP in the NL-In group
PG(34:1)	0.0040	2.3840	1.1160	1.0950	UP in the NL-In group
LPC(18:1)	0.0005	17.3260	8.1260	1.0923	UP in the NL-In group
DGTS(34:1)	0.0001	0.9620	0.4640	1.0519	UP in the NL-In group
PC(34:3)	0.0011	20.9920	10.6120	0.9841	-
DGDG(38:7)	0.0100	0.4540	0.2300	0.9811	-
PC(38:6)	0.0005	8.9560	4.6000	0.9612	-
PC(35:3)	0.0017	1.3020	0.6740	0.9499	-
DGDG(35:5)	0.0002	1.8000	0.9420	0.9342	-
SQDG(34:2)	0.0248	0.2600	0.1420	0.8726	-
MGTS(18:1)	0.0000	1.3860	0.7840	0.8220	-
PE(40:9)	0.0053	1.7380	0.9840	0.8207	-
PG(33:1)	0.0471	0.1200	0.0700	0.7776	-
DGTS(38:7)	0.0002	3.5380	2.0980	0.7539	-
PC(37:3)	0.0201	0.1700	0.1080	0.6545	-
PC(36:4(OH))	0.0069	0.3260	0.2080	0.6483	-

DGDG(30:1)	0.0160	1.7260	1.1240	0.6188	-
Cer(d32:2)	0.0105	4.8720	3.3880	0.5241	-
PE(38:6)	0.0024	1.6940	1.1860	0.5143	-
SQDG(32:3)	0.2420	0.0940	0.0660	0.5102	-
DGTS(34:2)	0.0003	1.5800	1.1140	0.5042	-
LPE(18:2)	0.0515	1.2000	0.8520	0.4941	-
MGDG(40:10)	0.0223	15.2520	10.9020	0.4844	-
MGTS(17:2)	0.0047	0.0920	0.0660	0.4792	-
PG(35:5)	0.2690	0.8080	0.5820	0.4733	-
LPC(18:2)	0.0453	12.9020	9.3520	0.4642	-
DGTS(34:3)	0.0001	0.9980	0.7240	0.4631	-
LPE(18:1)	0.0783	1.5140	1.1060	0.4530	-
PC(34:1)	0.0217	16.5440	12.1700	0.4430	-
DGTS(36:4)	0.1020	9.3280	7.0480	0.4044	-
PC(35:2)	0.0223	0.9100	0.6920	0.3951	-
PG(36:5)	0.1180	90.5540	72.9260	0.3123	-
SQDG(32:2)	0.4520	2.6020	2.1260	0.2915	-
DGDG(34:5)	0.2990	9.8240	8.1740	0.2653	-
DGDG(38:6)	0.5330	0.1560	0.1300	0.2630	-
PE(38:7)	0.1130	0.5180	0.4360	0.2486	-
MGTS(18:2)	0.1270	1.7540	1.5060	0.2199	-
DGTS(33:2)	0.2310	0.1500	0.1300	0.2065	-
PC(40:9)	0.2950	3.0700	2.6680	0.2025	-
DGDG(36:7)	0.3200	2.2820	1.9840	0.2019	-
DGTS(32:1)	0.1730	7.5800	6.6400	0.1910	-
PC(35:1)	0.3380	0.2780	0.2480	0.1647	-
DGDG(36:5)	0.5340	35.5000	31.9760	0.1508	-
LPG(16:0)	0.7160	0.2420	0.2180	0.1507	-
PI(34:5)	0.8060	0.0484	0.0444	0.1244	-
SQDG(30:0)	0.8060	13.1280	12.1460	0.1122	-
Cer(d32:1)	0.3480	0.8760	0.8220	0.0918	-
PC(36:4)	0.5120	9.5820	9.0140	0.0882	-
PE(40:10)	0.5960	0.3380	0.3200	0.0790	-
LPE(20:2)	0.9360	0.0500	0.0480	0.0589	-
PE(30:3)	0.0289	0.7460	0.7180	0.0552	-
PC(35:4)	0.7150	0.7280	0.7080	0.0402	-
MGTS(20:4)	0.8460	4.1660	4.0740	0.0322	-
LPE(20:4)	0.9670	19.3560	19.1220	0.0175	-
DGTS(38:10)	0.9190	0.1240	0.1240	0.0000	-
PG(32:1)	0.8900	17.0660	17.4540	-0.0324	-
PE(40:7)	0.7960	0.6840	0.7220	-0.0780	-
DGTS(34:4)	0.5080	4.1600	4.4340	-0.0920	-
PC(38:7)	0.4350	3.3820	3.6100	-0.0941	-
SQDG(28:0)	0.6350	0.4300	0.4640	-0.1098	-
DGDG(32:5)	0.3540	0.2660	0.2900	-0.1246	-
DGTS(36:7)	0.0176	0.6780	0.7440	-0.1340	-
SQDG(34:1)	0.5030	0.6760	0.7440	-0.1383	-
PI(32:2)	0.5040	3.6000	3.9640	-0.1390	-
PC(30:1)	0.3230	0.7740	0.8540	-0.1419	-
PC(37:4)	0.3180	0.2320	0.2620	-0.1754	-
PC(32:2)	0.3480	8.2800	9.3640	-0.1775	-

PC(33:3)	0.3600	0.3000	0.3440	-0.1974	-
PC(34:4)	0.0955	3.9120	4.5460	-0.2167	-
PC(37:6)	0.1880	0.5040	0.5920	-0.2322	-
PG(34:2)	0.7710	1.4820	1.7560	-0.2447	-
MGTS(18:5)	0.0993	0.3480	0.4260	-0.2918	-
LPC(20:4)	0.1090	4.4740	5.5860	-0.3203	-
DGTS(32:3)	0.0352	0.3340	0.4180	-0.3237	-
DGTS(40:9)	0.0091	7.4940	9.5180	-0.3449	-
DGTS(38:8)	0.0230	4.3040	5.4900	-0.3511	-
PC(37:5)	0.0291	0.4820	0.6260	-0.3771	-
DGTS(38:9)	0.0394	0.9940	1.2940	-0.3805	-
PE(38:9)	0.0806	0.1520	0.2020	-0.4103	-
PC(32:1)	0.0379	7.1160	9.5420	-0.4232	-
PE(36:6)	0.0482	0.5000	0.6720	-0.4265	-
DGMG(16:0)	0.0219	0.6660	0.9060	-0.4440	-
PI(33:1)	0.1680	0.5880	0.8080	-0.4585	-
DGTS(32:2)	0.0139	2.6540	3.6680	-0.4668	-
PC(30:3)	0.0494	0.5480	0.7600	-0.4718	-
PE(36:5)	0.0307	0.9160	1.2860	-0.4895	-
PC(33:2)	0.0301	0.6060	0.8520	-0.4915	-
PC(37:2)	0.1220	0.2600	0.3680	-0.5012	-
PE(38:8)	0.0248	0.3300	0.4700	-0.5102	-
PC(36:6)	0.0187	7.3800	10.5700	-0.5183	-
DGTS(33:1)	0.1470	0.0680	0.0980	-0.5272	-
DGDG(36:6)	0.0080	13.0940	19.0160	-0.5383	-
PC(32:3)	0.0175	1.0940	1.5960	-0.5448	-
PE(30:0)	0.1040	0.0300	0.0440	-0.5525	-
DGTS(36:6)	0.0024	3.7500	5.5060	-0.5541	-
SQDG(32:1)	0.0073	60.7280	89.8720	-0.5655	-
LPE(16:2)	0.1030	0.1140	0.1720	-0.5934	-
DGTS(36:5)	0.0107	20.3960	31.1300	-0.6100	-
MGTS(16:2)	0.0004	0.3440	0.5280	-0.6181	-
PC(36:5)	0.0034	13.8980	21.9920	-0.6621	-
PC(37:7)	0.0013	0.2280	0.3680	-0.6907	-
PI(40:10)	0.1570	0.0148	0.0240	-0.6974	-
DGTS(32:5)	0.0026	0.1340	0.2180	-0.7021	-
PC(34:5)	0.0026	2.1460	3.5780	-0.7375	-
SQDG(31:1)	0.0305	0.5340	0.8940	-0.7434	-
MGTS(18:3)	0.0031	2.6640	4.6040	-0.7893	-
DGTS(30:2)	0.0001	0.0800	0.1400	-0.8074	-
PE(36:4)	0.0018	0.2060	0.3620	-0.8133	-
PG(31:0)	0.0074	0.5860	1.0500	-0.8414	-
PC(36:7)	0.0017	0.6040	1.0900	-0.8517	-
PC(38:10)	0.0050	0.1040	0.1900	-0.8694	-
PC(32:4)	0.0017	0.3300	0.6080	-0.8816	-
SQDG(30:1)	0.0106	2.2040	4.0640	-0.8828	-
PC(34:6)	0.0022	0.2400	0.4440	-0.8875	-
MGTS(17:1)	0.0019	0.0560	0.1040	-0.8931	-
MGTS(18:4)	0.0001	2.4680	4.6360	-0.9095	-
DGTS(34:6)	0.0002	0.1240	0.2340	-0.9162	-
PE(36:7)	0.0010	0.0940	0.1780	-0.9211	-

PC(28:1)	0.0031	0.0200	0.0380	-0.9260	-
PC(40:10)	0.0012	0.8820	1.6820	-0.9313	-
LPE(20:5)	0.0004	5.7460	11.2220	-0.9657	-
PC(31:2)	0.0022	0.0300	0.0600	-1.0000	-
PG(34:5)	0.0044	0.6660	1.3700	-1.0406	DOWN in the NL-In group
PC(38:9)	0.0005	1.0700	2.2260	-1.0568	DOWN in the NL-In group
PI(32:1)	0.0002	19.0020	39.8100	-1.0670	DOWN in the NL-In group
PC(40:11)	0.0029	0.0720	0.1540	-1.0969	DOWN in the NL-In group
LPE(18:3)	0.0028	0.2200	0.4740	-1.1074	DOWN in the NL-In group
DGTS(32:4)	0.0001	0.5000	1.0820	-1.1137	DOWN in the NL-In group
LPC(18:3)	0.0002	1.1360	2.5200	-1.1495	DOWN in the NL-In group
PC(31:1)	0.0001	0.1040	0.2320	-1.1575	DOWN in the NL-In group
PG(32:2)	0.0037	0.6620	1.5540	-1.2311	DOWN in the NL-In group
LPC(20:1)	0.0343	0.0084	0.0200	-1.2515	DOWN in the NL-In group
DGTS(30:3)	0.0000	0.0460	0.1100	-1.2578	DOWN in the NL-In group
PE(34:3)	0.0001	0.0680	0.1640	-1.2701	DOWN in the NL-In group
PE(40:1)	0.0000	0.1300	0.3180	-1.2905	DOWN in the NL-In group
DGTS(36:8)	0.0001	1.0960	2.6840	-1.2921	DOWN in the NL-In group
PG(30:1)	0.0204	0.8740	2.1720	-1.3133	DOWN in the NL-In group
PI-Cer(d18:1/14:1)	0.0329	0.8340	2.0760	-1.3157	DOWN in the NL-In group
DGTS(36:2)	0.0001	0.1660	0.4140	-1.3184	DOWN in the NL-In group
DGTS(34:5)	0.0003	6.6660	16.7580	-1.3300	DOWN in the NL-In group
PE(34:4)	0.0000	0.1680	0.4260	-1.3424	DOWN in the NL-In group
MGTS(16:0)	0.0004	5.1420	13.0520	-1.3439	DOWN in the NL-In group
DGMG(16:1)	0.0057	0.2020	0.5160	-1.3530	DOWN in the NL-In group
DGMG(14:0)	0.0204	0.0320	0.0820	-1.3576	DOWN in the NL-In group
PE(32:3)	0.0072	0.0240	0.0640	-1.4150	DOWN in the NL-In group
PE(34:6)	0.0333	0.0104	0.0280	-1.4288	DOWN in the NL-In group
DGTS(30:1)	0.0000	1.5620	4.2260	-1.4359	DOWN in the NL-In group
PC(38:8)	0.0003	2.0900	5.8080	-1.4745	DOWN in the NL-In group
MGTS(16:1)	0.0000	4.8680	13.5860	-1.4807	DOWN in the NL-In group
DGTS(28:0)	0.0001	0.1380	0.3860	-1.4839	DOWN in the NL-In group
DGTS(40:10)	0.0000	10.4800	29.6180	-1.4988	DOWN in the NL-In group
MGTS(20:5)	0.0000	38.0560	109.4280	-1.5238	DOWN in the NL-In group
DGTS(37:5)	0.0000	0.1680	0.4900	-1.5443	DOWN in the NL-In group
LPC(17:2)	0.0000	0.0720	0.2140	-1.5715	DOWN in the NL-In group
DGDG(40:10)	0.0008	1.4840	4.4520	-1.5850	DOWN in the NL-In group
PG(36:5(OH))	0.0000	5.2680	16.1460	-1.6158	DOWN in the NL-In group
LPC(16:1)	0.0000	2.9500	9.1600	-1.6346	DOWN in the NL-In group
PE(30:1)	0.0020	0.0540	0.1700	-1.6545	DOWN in the NL-In group
LPE(18:4)	0.0040	0.2500	0.8120	-1.6996	DOWN in the NL-In group
PE(32:4)	0.0024	0.0220	0.0720	-1.7105	DOWN in the NL-In group
PE(32:1)	0.0000	0.3020	1.0220	-1.7588	DOWN in the NL-In group
MGTS(16:3)	0.0001	0.7640	2.6020	-1.7680	DOWN in the NL-In group
LPC(18:4)	0.0000	0.5400	1.8460	-1.7734	DOWN in the NL-In group
SQDG(36:5)	0.0024	0.0740	0.2580	-1.8018	DOWN in the NL-In group
PI(34:1)	0.0081	2.9220	10.4280	-1.8354	DOWN in the NL-In group
LPC(17:1)	0.0003	0.1160	0.4300	-1.8902	DOWN in the NL-In group
LPE(16:1)	0.0036	0.6580	2.4480	-1.8954	DOWN in the NL-In group
LPC(20:5)	0.0000	4.3520	16.2620	-1.9018	DOWN in the NL-In group
PI(34:2)	0.0060	0.6300	2.4660	-1.9687	DOWN in the NL-In group

PG(36:2)	0.0001	0.7780	3.1740	-2.0285	DOWN in the NL-In group
PG(31:1)	0.0116	0.1020	0.4240	-2.0555	DOWN in the NL-In group
PE(36:8)	0.0004	0.0340	0.1420	-2.0623	DOWN in the NL-In group
LPE(20:3)	0.0012	0.4180	1.7520	-2.0674	DOWN in the NL-In group
MGTS(14:0)	0.0000	1.5120	6.5780	-2.1212	DOWN in the NL-In group
PE(34:5)	0.0000	0.1260	0.5720	-2.1826	DOWN in the NL-In group
LPC(18:5)	0.0000	0.0420	0.1960	-2.2224	DOWN in the NL-In group
LPC(16:0)	0.0002	1.5440	7.3120	-2.2436	DOWN in the NL-In group
DGTS(28:1)	0.0050	0.0084	0.0400	-2.2515	DOWN in the NL-In group
LPE(16:0)	0.0001	0.1120	0.5380	-2.2641	DOWN in the NL-In group
LPC(16:2)	0.0000	0.2220	1.0680	-2.2663	DOWN in the NL-In group
LPC(17:0)	0.0001	0.0100	0.0500	-2.3219	DOWN in the NL-In group
PC(32:5)	0.0000	0.0280	0.1420	-2.3424	DOWN in the NL-In group
PC(36:8)	0.0000	0.0940	0.5020	-2.4170	DOWN in the NL-In group
LPE(16:3)	0.0063	0.0104	0.0560	-2.4288	DOWN in the NL-In group
PE(34:2)	0.0002	0.2300	1.2720	-2.4674	DOWN in the NL-In group
PE(32:2)	0.0000	0.2640	1.4780	-2.4850	DOWN in the NL-In group
LPC(14:0)	0.0000	0.0800	0.4500	-2.4919	DOWN in the NL-In group
DGMG(20:5)	0.0041	0.0780	0.4480	-2.5220	DOWN in the NL-In group
PG(36:6)	0.0000	6.8280	41.8060	-2.6142	DOWN in the NL-In group
LPE(14:0)	0.0002	0.0140	0.0860	-2.6189	DOWN in the NL-In group
MGTS(14:1)	0.0000	0.0120	0.0760	-2.6630	DOWN in the NL-In group
MGTS(15:0)	0.0000	0.0760	0.4960	-2.7063	DOWN in the NL-In group
PC(34:7)	0.0001	0.0120	0.0800	-2.7370	DOWN in the NL-In group
PE(36:3)	0.0000	0.0340	0.2780	-3.0315	DOWN in the NL-In group
PI(30:1)	0.0002	0.4220	4.0100	-3.2483	DOWN in the NL-In group
LPC(22:6)	0.0001	0.0160	0.1560	-3.2854	DOWN in the NL-In group
LPC(16:3)	0.0000	0.0260	0.2540	-3.2882	DOWN in the NL-In group
LPC(22:5)	0.0016	0.0068	0.0720	-3.4044	DOWN in the NL-In group
PE(36:2)	0.0000	0.0520	0.6240	-3.5850	DOWN in the NL-In group
LPC(20:3)	0.0000	0.0760	0.9420	-3.6317	DOWN in the NL-In group
MGTS(16:4)	0.0002	0.0036	0.0620	-4.1062	DOWN in the NL-In group
PI(34:3)	0.0014	0.0080	0.2480	-4.9542	DOWN in the NL-In group
PI(36:6)	0.0014	0.0060	0.2000	-5.0589	DOWN in the NL-In group

Table S5.16. Results of Kruskal-Wallis test (FDR adjusted) for statistically significant differences of polar lipid classes between the groups. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

Variable	**.y.**	**n**	**statistic**	**df**	**p**	**method**	**p.adj**
LPC	value	20	17.8571	3	0.0004710	Kruskal-Wallis	**0.0041600**
MGMG	value	20	17.6492	3	0.0005200	Kruskal-Wallis	**0.0041600**
DGTS	value	20	15.6629	3	0.0013300	Kruskal-Wallis	**0.0042560**
Cer	value	20	15.8343	3	0.0012300	Kruskal-Wallis	**0.0042560**

MGDG	value	20	16.0743	3	0.0010900	Kruskal-Wallis	**0.0042560**
PI-Cer	value	20	15.1943	3	0.0016600	Kruskal-Wallis	**0.0043429**
SQDG	value	20	14.9086	3	0.0019000	Kruskal-Wallis	**0.0043429**
DGMG	value	20	14.3444	3	0.0024700	Kruskal-Wallis	**0.0043911**
MGTS	value	20	14.4514	3	0.0023500	Kruskal-Wallis	**0.0043911**
LPG	value	20	13.8684	3	0.0030900	Kruskal-Wallis	**0.0049440**
DGDG	value	20	13.5486	3	0.0035900	Kruskal-Wallis	**0.0052218**
LPE	value	20	13.2171	3	0.0041900	Kruskal-Wallis	**0.0055867**
PI	value	20	11.9600	3	0.0075200	Kruskal-Wallis	**0.0092554**
PG	value	20	11.5257	3	0.0092000	Kruskal-Wallis	**0.0105143**
PC	value	20	9.1486	3	0.0274000	Kruskal-Wallis	**0.0292267**
PE	value	20	3.6971	3	0.2960000	Kruskal-Wallis	0.2960000

Table S5.17. Results of Dunn's multiple comparison post-hoc test (FDR adjusted) of significant lipid classes (*q* values < 0.05) identified in the total lipid extracts of *N. oceanica* (NO) and *N. limnetica* (NL) grown outdoors (Out) and indoors (In). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; DGTS, diacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4´-(N,N,N-trimethyl) homoserine; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; Cer, ceramide and PI-Cer, inositolphosphoceramide.

Variable	.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
DGDG	value	1.NL.In	2.NL.Out	5	5	-1.8174	0.0691595	0.1324331	ns
DGDG	value	1.NL.In	3.NO.In	5	5	-0.6414	0.5212453	0.5864010	ns
DGDG	value	1.NL.In	4.NO.Out	5	5	-3.4209	0.0006240	0.0070205	**
DGDG	value	2.NL.Out	3.NO.In	5	5	1.1759	0.2396151	0.3125414	ns
DGDG	value	2.NL.Out	4.NO.Out	5	5	-1.6036	0.1088094	0.1718044	ns
DGDG	value	3.NO.In	4.NO.Out	5	5	-2.7795	0.0054440	0.0222708	*
DGMG	value	1.NL.In	2.NL.Out	5	5	1.5780	0.1145591	0.1747512	ns
DGMG	value	1.NL.In	3.NO.In	5	5	-1.6048	0.1085434	0.1718044	ns
DGMG	value	1.NL.In	4.NO.Out	5	5	-1.6850	0.0919860	0.1505226	ns
DGMG	value	2.NL.Out	3.NO.In	5	5	-3.1828	0.0014586	0.0100978	*
DGMG	value	2.NL.Out	4.NO.Out	5	5	-3.2630	0.0011022	0.0083386	**
DGMG	value	3.NO.In	4.NO.Out	5	5	-0.0802	0.9360474	0.9573212	ns
DGTS	value	1.NL.In	2.NL.Out	5	5	1.9777	0.0479588	0.1003789	ns
DGTS	value	1.NL.In	3.NO.In	5	5	1.8708	0.0613688	0.1227377	ns
DGTS	value	1.NL.In	4.NO.Out	5	5	3.9555	0.0000764	0.0013749	**
DGTS	value	2.NL.Out	3.NO.In	5	5	-0.1069	0.9148647	0.9464118	ns
DGTS	value	2.NL.Out	4.NO.Out	5	5	1.9777	0.0479588	0.1003789	ns
DGTS	value	3.NO.In	4.NO.Out	5	5	2.0846	0.0371022	0.0927555	ns
Cer	value	1.NL.In	2.NL.Out	5	5	-2.2450	0.0247685	0.0743055	ns
Cer	value	1.NL.In	3.NO.In	5	5	-1.8174	0.0691595	0.1324331	ns
Cer	value	1.NL.In	4.NO.Out	5	5	-3.9555	0.0000764	0.0013749	**
Cer	value	2.NL.Out	3.NO.In	5	5	0.4276	0.6689293	0.7341907	ns
Cer	value	2.NL.Out	4.NO.Out	5	5	-1.7105	0.0871786	0.1480392	ns
Cer	value	3.NO.In	4.NO.Out	5	5	-2.1381	0.0325094	0.0860544	ns
LPC	value	1.NL.In	2.NL.Out	5	5	1.3363	0.1814492	0.2512374	ns
LPC	value	1.NL.In	3.NO.In	5	5	-1.3363	0.1814492	0.2512374	ns

LPC	value	1.NL.In	4.NO.Out	5	5	-2.6726	0.0075263	0.0270947	*
LPC	value	2.NL.Out	3.NO.In	5	5	-2.6726	0.0075263	0.0270947	*
LPC	value	2.NL.Out	4.NO.Out	5	5	-4.0089	0.0000610	0.0013749	**
LPC	value	3.NO.In	4.NO.Out	5	5	-1.3363	0.1814492	0.2512374	ns
LPE	value	1.NL.In	2.NL.Out	5	5	1.2829	0.1995432	0.2721044	ns
LPE	value	1.NL.In	3.NO.In	5	5	-1.7639	0.0777447	0.1399405	ns
LPE	value	1.NL.In	4.NO.Out	5	5	-1.7639	0.0777447	0.1399405	ns
LPE	value	2.NL.Out	3.NO.In	5	5	-3.0468	0.0023131	0.0122457	*
LPE	value	2.NL.Out	4.NO.Out	5	5	-3.0468	0.0023131	0.0122457	*
LPE	value	3.NO.In	4.NO.Out	5	5	0.0000	1.0000000	1.0000000	ns
MGDG	value	1.NL.In	2.NL.Out	5	5	-2.0312	0.0422362	0.0974681	ns
MGDG	value	1.NL.In	3.NO.In	5	5	-1.9777	0.0479588	0.1003789	ns
MGDG	value	1.NL.In	4.NO.Out	5	5	-4.0089	0.0000610	0.0013749	**
MGDG	value	2.NL.Out	3.NO.In	5	5	0.0535	0.9573716	0.9681286	ns
MGDG	value	2.NL.Out	4.NO.Out	5	5	-1.9777	0.0479588	0.1003789	ns
MGDG	value	3.NO.In	4.NO.Out	5	5	-2.0312	0.0422362	0.0974681	ns
MGMG	value	1.NL.In	2.NL.Out	5	5	-2.7312	0.0063104	0.0246927	*
MGMG	value	1.NL.In	3.NO.In	5	5	-1.3388	0.1806276	0.2512374	ns
MGMG	value	1.NL.In	4.NO.Out	5	5	-3.9629	0.0000740	0.0013749	**
MGMG	value	2.NL.Out	3.NO.In	5	5	1.3924	0.1638079	0.2416838	ns
MGMG	value	2.NL.Out	4.NO.Out	5	5	-1.2317	0.2180540	0.2897487	ns
MGMG	value	3.NO.In	4.NO.Out	5	5	-2.6241	0.0086879	0.0300735	*
MGTS	value	1.NL.In	2.NL.Out	5	5	3.6882	0.0002258	0.0033876	**
MGTS	value	1.NL.In	3.NO.In	5	5	1.7639	0.0777447	0.1399405	ns
MGTS	value	1.NL.In	4.NO.Out	5	5	2.5657	0.0102965	0.0343218	*
MGTS	value	2.NL.Out	3.NO.In	5	5	-1.9243	0.0543194	0.1111078	ns
MGTS	value	2.NL.Out	4.NO.Out	5	5	-1.1225	0.2616511	0.3270639	ns
MGTS	value	3.NO.In	4.NO.Out	5	5	0.8018	0.4226781	0.4877055	ns
PC	value	1.NL.In	2.NL.Out	5	5	-2.8330	0.0046118	0.0197648	*
PC	value	1.NL.In	3.NO.In	5	5	-0.6949	0.4871310	0.5549594	ns
PC	value	1.NL.In	4.NO.Out	5	5	-1.7105	0.0871786	0.1480392	ns
PC	value	2.NL.Out	3.NO.In	5	5	2.1381	0.0325094	0.0860544	ns
PC	value	2.NL.Out	4.NO.Out	5	5	1.1225	0.2616511	0.3270639	ns
PC	value	3.NO.In	4.NO.Out	5	5	-1.0156	0.3098234	0.3768122	ns
LPG	value	1.NL.In	2.NL.Out	5	5	-0.1873	0.8514300	0.9015141	ns
LPG	value	1.NL.In	3.NO.In	5	5	3.0770	0.0020911	0.0122457	*
LPG	value	1.NL.In	4.NO.Out	5	5	1.4984	0.1340414	0.2010620	ns
LPG	value	2.NL.Out	3.NO.In	5	5	3.2643	0.0010975	0.0083386	**
LPG	value	2.NL.Out	4.NO.Out	5	5	1.6856	0.0918636	0.1505226	ns
LPG	value	3.NO.In	4.NO.Out	5	5	-1.5786	0.1144226	0.1747512	ns
PG	value	1.NL.In	2.NL.Out	5	5	2.1381	0.0325094	0.0860544	ns
PG	value	1.NL.In	3.NO.In	5	5	-1.1225	0.2616511	0.3270639	ns
PG	value	1.NL.In	4.NO.Out	5	5	-0.2673	0.7892680	0.8456443	ns
PG	value	2.NL.Out	3.NO.In	5	5	-3.2606	0.0011118	0.0083386	**
PG	value	2.NL.Out	4.NO.Out	5	5	-2.4054	0.0161569	0.0519330	ns
PG	value	3.NO.In	4.NO.Out	5	5	0.8552	0.3924205	0.4586733	ns
PI	value	1.NL.In	2.NL.Out	5	5	2.9399	0.0032835	0.0147756	*
PI	value	1.NL.In	3.NO.In	5	5	3.0468	0.0023131	0.0122457	*
PI	value	1.NL.In	4.NO.Out	5	5	2.0312	0.0422362	0.0974681	ns
PI	value	2.NL.Out	3.NO.In	5	5	0.1069	0.9148647	0.9464118	ns
PI	value	2.NL.Out	4.NO.Out	5	5	-0.9087	0.3635147	0.4304780	ns

PI	value	3.NO.In	4.NO.Out	5	5	-1.0156	0.3098234	0.3768122	ns
PI-Cer	value	1.NL.In	2.NL.Out	5	5	0.9090	0.3633343	0.4304780	ns
PI-Cer	value	1.NL.In	3.NO.In	5	5	2.9945	0.0027494	0.0137469	*
PI-Cer	value	1.NL.In	4.NO.Out	5	5	3.2618	0.0011070	0.0083386	**
PI-Cer	value	2.NL.Out	3.NO.In	5	5	2.0854	0.0370310	0.0927555	ns
PI-Cer	value	2.NL.Out	4.NO.Out	5	5	2.3528	0.0186335	0.0578280	ns
PI-Cer	value	3.NO.In	4.NO.Out	5	5	0.2674	0.7891906	0.8456443	ns
SQDG	value	1.NL.In	2.NL.Out	5	5	1.2294	0.2189212	0.2897487	ns
SQDG	value	1.NL.In	3.NO.In	5	5	3.4209	0.0006240	0.0070205	**
SQDG	value	1.NL.In	4.NO.Out	5	5	2.9399	0.0032835	0.0147756	*
SQDG	value	2.NL.Out	3.NO.In	5	5	2.1915	0.0284126	0.0824881	ns
SQDG	value	2.NL.Out	4.NO.Out	5	5	1.7105	0.0871786	0.1480392	ns
SQDG	value	3.NO.In	4.NO.Out	5	5	-0.4811	0.6304666	0.7005184	ns

Supplementary material of Chapter 6

Table S6.1. Exact mass measurements of the molecular ions identified by HILIC−ESI−MS in the total lipid extracts of *Spirulina* sp., *Chlorella vulgaris*, *Chlorococcum amblystomatis*, *Nannochloropsis oceanica*, *Scenedesmus obliquus*, *Phaeodactylum tricornutum* and *Tetraselmis chuii*. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains.

Lipid species (C:N)	Calculated *m/z*	Observed *m/z* in MS of the *Spirulina* sp. extracts	**Error (ppm)**	Observed *m/z* in MS of the *C. vulgaris* extracts	**Error (ppm)**	Observed *m/z* in MS of the *C. amblystomatis* extracts	**Error (ppm)**	Observed *m/z* in MS of the *N. oceanica* extracts	**Error (ppm)**	Observed *m/z* in MS of the *S. obliquus* extracts	**Error (ppm)**	Observed *m/z* in MS of the *P. tricornutum* extracts	**Error (ppm)**	Observed *m/z* in MS of the *T. chui* extracts	**Error (ppm)**
Cer(d32:1)	510.4886	-	-	-	-	510.4886	**0.0**	510.4885	**-0.2**	-	-	-	-	-	-
Cer(d32:2)	508.4730	-	-	-	-	508.4730	**0.0**	508.4726	**-0.8**	-	-	-	-	-	-
Cer(d35:0)	554.5512	-	-	554.5516	**0.7**	-	-	-	-	554.5512	**0.0**	-	-	-	-
DGDG(30:1)	880.5997	-	-	-	-	880.5997	**0.0**	880.5994	**-0.3**	-	-	880.5997	**0.0**	-	-
DGDG(30:2)	878.5841	-	-	-	-	-	-	878.5819	**-2.5**	-	-	878.5855	**1.6**	-	-
DGDG(32:0)	910.6466	910.6464	**-0.2**	910.6467	**0.1**	-	-	-	-	910.6421	**-4.9**	-	-	910.6427	**-4.3**
DGDG(32:1)	908.6310	908.6298	**-1.3**	908.6284	**-2.9**	908.6308	**-0.2**	908.6305	**-0.6**	908.6284	**-2.9**	908.6301	**-1.0**	908.6290	**-2.2**
DGDG(32:2)	906.6154	906.6146	**-0.9**	906.6154	**0.0**	906.6152	**-0.2**	906.6151	**-0.3**	906.6132	**-2.4**	906.6152	**-0.2**	906.6124	**-3.3**
DGDG(32:3)	904.5997	-	-	904.6027	**3.3**	904.6016	**2.1**	904.6023	**2.9**	904.6003	**0.7**	904.6003	**0.7**	904.5993	**-0.4**
DGDG(32:4)	902.5841	-	-	902.5852	**1.2**	902.5862	**2.3**	902.5867	**2.9**	902.5843	**0.2**	902.5852	**1.2**	902.5858	**1.9**
DGDG(32:5)	900.5684	-	-	900.5682	**-0.2**	900.5682	**-0.2**	900.5690	**0.7**	-	-	900.5684	**0.0**	900.5688	**0.4**
DGDG(32:6)	898.5528	-	-	-	-	898.5506	**-2.4**	-	-	898.5512	**-1.8**	898.5523	**-0.6**	-	-
DGDG(33:3)	918.6148	918.6166	**1.9**	918.6161	**1.4**	918.6165	**1.8**	918.6149	**0.1**	918.6151	**0.3**	-	-	918.6190	**4.6**
DGDG(34:1)	936.6623	-	-	936.6589	**-3.6**	936.6616	**-0.7**	936.6606	**-1.8**	936.6577	**-4.9**	-	-	936.6612	**-1.2**
DGDG(34:2)	934.6467	-	-	934.6463	**-0.4**	934.6443	**-2.6**	934.6457	**-1.1**	934.6444	**-2.5**	934.6463	**-0.4**	934.6451	**-1.7**
DGDG(34:3)	932.6310	932.6306	**-0.4**	932.6283	**-2.9**	932.6310	**0.0**	932.6308	**-0.2**	932.6308	**-0.2**	932.6307	**-0.3**	932.6304	**-0.6**
DGDG(34:4)	930.6154	930.6152	**-0.2**	930.6150	**-0.4**	930.6144	**-1.1**	-	-	930.6143	**-1.2**	930.6156	**0.2**	930.6145	**-1.0**
DGDG(34:5)	928.5997	928.5997	**0.0**	928.5981	**-1.7**	928.5992	**-0.5**	928.5994	**-0.3**	928.5981	**-1.7**	928.5997	**0.0**	928.5985	**-1.3**
DGDG(34:6)	926.5841	-	-	926.5843	**0.2**	926.5824	**-1.8**	926.5862	**2.3**	926.5833	**-0.9**	926.5840	**-0.1**	926.5830	**-1.2**

DGDG(34:7)	924.5684	-	-	-	-	924.5686	**0.2**	-	-	924.5688	**0.4**	924.5694	**1.1**	924.5666	**-1.9**
DGDG(34:8)	922.5528	-	-	-	-	922.5523	**-0.5**	-	-	-	-	922.5487	**-4.4**	922.5520	**-0.9**
DGDG(35:1)	950.6780	-	-	950.6771	**-0.9**	-	-	-	-	950.6785	**0.5**	-	-	950.6785	**0.5**
DGDG(35:3)	946.6467	946.6495	**3.0**	946.6477	**1.1**	946.6452	**-1.6**	-	-	946.6473	**0.6**	-	-	946.6489	**2.3**
DGDG(35:4)	944.6310	944.6312	**0.2**	944.6326	**1.6**	-	-	944.6299	**-1.2**	944.6321	**1.1**	-	-	944.6315	**0.5**
DGDG(35:5)	942.6154	942.6122	**-3.4**	942.6185	**3.3**	942.6152	**-0.2**	942.6159	**0.5**	942.6197	**4.6**	942.6185	**3.3**	942.6196	**4.5**
DGDG(36:1)	964.6937	-	-	-	-	-	-	-	-	964.6921	**-1.7**	-	-	964.6917	**-2.1**
DGDG(36:2)	962.6780	962.6738	**-4.4**	962.6757	**-2.4**	962.6757	**-2.4**	-	-	962.6776	**-0.4**	962.6766	**-1.5**	962.6736	**-4.6**
DGDG(36:3)	960.6623	960.6610	**-1.4**	960.6575	**-5.0**	-	-	-	-	960.6581	**-4.4**	-	-	960.6585	**-4.0**
DGDG(36:4)	958.6467	958.6441	**-2.7**	958.6462	**-0.5**	958.6429	**-4.0**	-	-	958.6431	**-3.8**	-	-	958.6422	**-4.7**
DGDG(36:5)	956.6310	956.6326	**1.7**	956.6308	**-0.2**	956.6302	**-0.8**	956.6301	**-0.9**	-	-	956.6303	**-0.7**	956.6313	**0.3**
DGDG(36:6)	954.6154	-	-	954.6157	**0.3**	954.6154	**0.0**	954.6151	**-0.3**	954.6153	**-0.1**	954.6134	**-2.1**	954.6151	**-0.3**
DGDG(36:7)	952.5997	-	-	952.5984	**-1.4**	952.5974	**-2.4**	952.5955	**-4.4**	952.5997	**0.0**	952.5999	**0.2**	952.5960	**-3.9**
DGDG(36:8)	950.5841	-	-	-	-	950.5819	**-2.3**	-	-	-	-	950.5830	**-1.2**	950.5830	**-1.2**
DGDG(36:9)	948.5684	-	-	-	-	-	-	-	-	-	-	948.5690	**0.6**	948.5681	**-0.3**
DGDG(37:2)	976.6937	-	-	976.6894	**-4.4**	-	-	-	-	976.6940	**0.4**	-	-	-	-
DGDG(38:6)	982.6467	-	-	-	-	982.6432	**-3.6**	982.6460	**-0.7**	-	-	982.6443	**-2.4**	982.6442	**-2.5**
DGDG(38:7)	980.6310	-	-	-	-	980.6319	**0.9**	980.6324	**1.4**	-	-	980.6311	**0.1**	-	-
DGDG(40:10)	1002.6154	-	-	-	-	1002.6158	**0.4**	1002.6159	**0.5**	-	-	1002.6159	**0.5**	-	-
DGMG(14:0)	644.3857	-	-	-	-	644.3842	**-2.3**	644.3859	**0.3**	-	-	-	-	-	-
DGMG(16:0)	672.4170	672.4164	**-0.9**	672.4173	**0.4**	672.4175	**0.7**	672.4173	**0.4**	672.4172	**0.3**	672.4172	**0.3**	672.4172	**0.3**
DGMG(16:1)	670.4014	670.4011	**-0.4**	-	-	670.4012	**-0.3**	670.4013	**-0.1**	-	-	670.4014	**0.0**	-	-
DGMG(16:2)	668.3857	668.3849	**-1.2**	668.3849	**-1.2**	668.3843	**-2.1**	-	-	-	-	668.3852	**-0.7**	-	-
DGMG(16:3)	666.3701	-	-	-	-	666.3700	**-0.2**	-	-	-	-	666.3700	**-0.2**	-	-
DGMG(16:4)	664.3544	-	-	-	-	664.3546	**0.3**	-	-	-	-	-	-	-	-
DGMG(18:1)	698.4327	-	-	-	-	698.4331	**0.6**	-	-	-	-	698.4332	**0.7**	698.4332	**0.7**
DGMG(18:2)	696.4170	696.4143	**-3.9**	696.4161	**-1.3**	696.4161	**-1.3**	696.4171	**0.1**	696.4146	**-3.4**	696.4170	**0.0**	696.4162	**-1.1**
DGMG(18:3)	694.4014	694.4012	**-0.3**	694.4012	**-0.3**	694.4013	**-0.1**	-	-	694.4012	**-0.3**	694.4017	**0.5**	694.4007	**-1.0**
DGMG(18:4)	692.3857	-	-	-	-	692.3857	**0.0**	-	-	-	-	-	-	692.3856	**-0.1**
DGMG(20:0)	728.4796	-	-	-	-	-	-	-	-	728.4809	**1.7**	-	-	-	-
DGMG(20:1)	726.4640	-	-	-	-	-	-	-	-	726.4656	**2.2**	-	-	-	-
DGMG(20:5)	718.4014	-	-	-	-	718.4013	**-0.1**	718.4010	**-0.6**	-	-	718.4018	**0.6**	-	-
DGTA(30:1)	682.5622	-	-	-	-	-	-	-	-	-	-	682.5618	**-0.6**	682.5622	**0.0**
DGTA(30:2)	680.5465	-	-	-	-	-	-	-	-	-	-	680.5458	**-1.0**	-	-
DGTA(30:4)	676.5152	-	-	-	-	-	-	-	-	-	-	676.5151	**-0.1**	676.5152	**0.0**
DGTA(32:1)	710.5935	-	-	-	-	-	-	-	-	-	-	710.5915	**-2.8**	710.5928	**-1.0**
DGTA(32:2)	708.5778	-	-	-	-	-	-	-	-	-	-	708.5776	**-0.3**	708.5774	**-0.6**

DGTA(32:3)	706.5622	-	-	-	-	-	-	-	-	-	-	706.5616	**-0.8**	706.5587	**-5.0**
DGTA(32:4)	704.5465	-	-	-	-	-	-	-	-	-	-	704.5448	**-2.4**	704.5464	**-0.1**
DGTA(32:5)	702.5309	-	-	-	-	-	-	-	-	-	-	702.5275	**-4.8**	702.5293	**-2.2**
DGTA(32:6)	700.5152	-	-	-	-	-	-	-	-	-	-	700.5122	**-4.3**	700.5142	**-1.5**
DGTA(32:7)	698.4996	-	-	-	-	-	-	-	-	-	-	698.4973	**-3.3**	698.4971	**-3.6**
DGTA(34:1)	738.6248	-	-	-	-	-	-	-	-	-	-	738.6212	**-4.9**	738.6232	**-2.2**
DGTA(34:2)	736.6091	-	-	-	-	-	-	-	-	-	-	736.6079	**-1.6**	736.6066	**-3.4**
DGTA(34:3)	734.5935	-	-	-	-	-	-	-	-	-	-	734.5932	**-0.4**	734.5924	**-1.5**
DGTA(34:4)	732.5778	-	-	-	-	-	-	-	-	-	-	732.5767	**-1.5**	732.5771	**-1.0**
DGTA(34:5)	730.5622	-	-	-	-	-	-	-	-	-	-	730.5611	**-1.5**	730.5617	**-0.7**
DGTA(34:6)	728.5465	-	-	-	-	-	-	-	-	-	-	728.5444	**-2.9**	728.5440	**-3.4**
DGTA(34:7)	726.5309	-	-	-	-	-	-	-	-	-	-	726.5289	**-2.7**	726.5283	**-3.5**
DGTA(34:8)	724.5152	-	-	-	-	-	-	-	-	-	-	724.5131	**-2.9**	724.5124	**-3.9**
DGTA(36:10)	748.5152	-	-	-	-	-	-	-	-	-	-	748.5123	**-3.9**	748.5124	**-3.8**
DGTA(36:2)	764.6404	-	-	-	-	-	-	-	-	-	-	764.6400	**-0.5**	764.6396	**-1.0**
DGTA(36:3)	762.6248	-	-	-	-	-	-	-	-	-	-	762.6211	**-4.9**	-	-
DGTA(36:4)	760.6091	-	-	-	-	-	-	-	-	760.6079	**-1.6**	760.6071	**-2.6**	760.6056	**-4.6**
DGTA(36:5)	758.5935	-	-	-	-	-	-	-	-	758.5936	**0.1**	758.5919	**-2.1**	758.5932	**-0.4**
DGTA(36:6)	756.5778	-	-	-	-	-	-	-	-	-	-	756.5772	**-0.8**	756.5756	**-2.9**
DGTA(36:7)	754.5622	-	-	-	-	-	-	-	-	-	-	754.5612	**-1.3**	754.5593	**-3.8**
DGTA(36:8)	752.5465	-	-	-	-	-	-	-	-	-	-	752.5439	**-3.5**	752.5438	**-3.6**
DGTA(36:9)	750.5309	-	-	-	-	-	-	-	-	-	-	750.5301	**-1.0**	750.5305	**-0.5**
DGTA(38:5)	786.6248	-	-	-	-	-	-	-	-	-	-	786.6219	**-3.7**	786.6214	**-4.3**
DGTA(38:6)	784.6091	-	-	-	-	-	-	-	-	-	-	784.6053	**-4.8**	784.6086	**-0.6**
DGTA(38:7)	782.5935	-	-	-	-	-	-	-	-	782.5914	**-2.7**	782.5921	**-1.8**	782.5896	**-5.0**
DGTA(38:8)	780.5778	-	-	-	-	-	-	-	-	780.5744	**-4.4**	780.5769	**-1.2**	780.5740	**-4.9**
DGTA(38:9)	778.5622	-	-	-	-	-	-	-	-	-	-	778.5601	**-2.7**	778.5607	**-1.9**
DGTA(40:10)	804.5778	-	-	-	-	-	-	804.5771	**-0.9**	804.5787	**1.1**	804.5759	**-2.4**	804.5773	**-0.6**
DGTA(40:11)	802.5622	-	-	-	-	-	-	-	-	-	-	802.5605	**-2.1**	802.5588	**-4.2**
DGTA(40:6)	812.6404	-	-	-	-	-	-	-	-	-	-	812.6401	**-0.4**	812.6397	**-0.9**
DGTA(40:7)	810.6248	-	-	-	-	-	-	-	-	-	-	810.6220	**-3.5**	-	-
DGTA(40:8)	808.6091	-	-	-	-	-	-	-	-	808.6066	**-3.1**	808.6077	**-1.7**	-	-
DGTA(40:9)	806.5935	-	-	-	-	-	-	-	-	806.5916	**-2.4**	806.5905	**-3.7**	806.5904	**-3.8**
DGTA(42:10)	832.6091	-	-	-	-	-	-	-	-	-	-	832.6055	**-4.4**	832.6084	**-0.9**
DGTA(42:11)	830.5935	-	-	-	-	-	-	-	-	-	-	830.5930	**-0.6**	-	-
DGTS(28:0)	656.5465	656.5481	**2.4**	656.5467	**0.3**	656.5453	**-1.8**	656.5474	**1.4**	-	-	-	-	656.5460	**-0.8**
DGTS(28:1)	654.5309	654.5307	**-0.3**	654.5302	**-1.1**	654.5307	**-0.3**	654.5308	**-0.2**	-	-	-	-	-	-
DGTS(29:0)	670.5616	670.5623	**1.1**	670.5627	**1.7**	-	-	-	-	-	-	670.5624	**1.2**	-	-

DGTS(30:0)	684.5778	684.5782	**0.6**	684.5781	**0.4**	-	-	-	-	684.5746	**-4.7**	684.5783	**0.7**	684.5771	**-1.0**
DGTS(30:1)	682.5622	682.5621	**-0.1**	682.5629	**1.0**	682.5621	**-0.1**	682.5620	**-0.3**	682.5622	**0.0**	682.5619	**-0.4**	682.5617	**-0.7**
DGTS(30:2)	680.5465	-	-	-	-	680.5450	**-2.2**	680.5458	**-1.0**	-	-	680.5441	**-3.5**	-	-
DGTS(30:3)	678.5309	678.5302	**-1.0**	678.5317	**1.2**	678.5309	**0.0**	678.5308	**-0.1**	678.5300	**-1.3**	-	-	678.5311	**0.3**
DGTS(30:4)	676.5152	-	-	-	-	676.5155	**0.4**	676.5123	**-4.3**	676.5156	**0.6**	-	-	-	-
DGTS(30:5)	674.4996	-	-	-	-	674.4995	**-0.1**	-	-	-	-	-	-	-	-
DGTS(31:1)	696.5773	696.5782	**1.3**	696.5781	**1.2**	696.5781	**1.2**	696.5777	**0.6**	696.5778	**0.8**	696.5780	**1.0**	696.5765	**-1.1**
DGTS(31:3)	692.5459	692.5449	**-1.4**	692.5453	**-0.8**	692.5463	**0.6**	-	-	692.5460	**0.2**	-	-	-	-
DGTS(32:0)	712.6091	712.6087	**-0.6**	712.6086	**-0.7**	-	-	-	-	-	-	-	-	-	-
DGTS(32:1)	710.5935	710.5932	**-0.4**	710.5945	**1.4**	710.5934	**-0.1**	710.5930	**-0.7**	710.5934	**-0.1**	710.5930	**-0.7**	710.5926	**-1.3**
DGTS(32:2)	708.5778	708.5782	**0.6**	708.5772	**-0.8**	708.5774	**-0.6**	708.5779	**0.1**	708.5747	**-4.4**	708.5775	**-0.4**	708.5773	**-0.7**
DGTS(32:3)	706.5622	706.5605	**-2.4**	706.5607	**-2.1**	706.5613	**-1.3**	706.5617	**-0.7**	706.5610	**-1.7**	706.5615	**-1.0**	706.5596	**-3.7**
DGTS(32:4)	704.5465	704.5443	**-3.1**	-	-	704.5468	**0.4**	704.5446	**-2.7**	704.5465	**0.0**	704.5445	**-2.8**	704.5456	**-1.3**
DGTS(32:5)	702.5309	-	-	-	-	702.5308	**-0.1**	702.5297	**-1.7**	702.5310	**0.1**	-	-	-	-
DGTS(32:6)	700.5152	-	-	-	-	700.5151	**-0.1**	700.5119	**-4.7**	700.5152	**0.0**	-	-	-	-
DGTS(32:7)	698.4996	-	-	-	-	698.4995	**-0.1**	-	-	698.4998	**0.3**	-	-	-	-
DGTS(32:8)	696.4839	-	-	-	-	696.4837	**-0.3**	-	-	696.4870	**4.5**	-	-	-	-
DGTS(33:1)	724.6091	724.6089	**-0.3**	724.6090	**-0.1**	724.6056	**-4.8**	724.6075	**-2.2**	724.6090	**-0.1**	724.6086	**-0.7**	724.6079	**-1.7**
DGTS(33:2)	722.5929	722.5937	**1.1**	722.5922	**-1.0**	722.5920	**-1.2**	722.5932	**0.4**	722.5905	**-3.3**	722.5923	**-0.8**	722.5893	**-5.0**
DGTS(33:3)	720.5767	720.5767	**0.0**	720.5776	**1.2**	720.5767	**0.0**	720.5767	**0.0**	720.5761	**-0.8**	720.5782	**2.1**	720.5747	**-2.8**
DGTS(33:4)	718.5605	718.5599	**-0.8**	718.5613	**1.1**	718.5619	**1.9**	718.5595	**-1.4**	718.5621	**2.2**	718.5603	**-0.3**	718.5587	**-2.5**
DGTS(34:1)	738.6248	738.6243	**-0.7**	738.6240	**-1.1**	738.6243	**-0.7**	738.6222	**-3.5**	738.6235	**-1.8**	738.6240	**-1.1**	738.6235	**-1.8**
DGTS(34:2)	736.6091	736.6091	**0.0**	736.6090	**-0.1**	736.6057	**-4.6**	736.6090	**-0.1**	736.6056	**-4.8**	736.6089	**-0.3**	736.6082	**-1.2**
DGTS(34:3)	734.5935	734.5924	**-1.5**	734.5927	**-1.1**	734.5924	**-1.5**	734.5929	**-0.8**	734.5907	**-3.8**	734.5919	**-2.2**	734.5925	**-1.4**
DGTS(34:4)	732.5778	732.5759	**-2.6**	732.5768	**-1.4**	732.5779	**0.1**	732.5756	**-3.0**	732.5780	**0.3**	732.5756	**-3.0**	732.5757	**-2.9**
DGTS(34:5((OH))	746.5571	-	-	-	-	746.5568	**-0.4**	746.5589	**2.4**	746.5568	**-0.4**	-	-	746.5579	**1.1**
DGTS(34:5)	730.5622	730.5604	**-2.5**	730.5636	**1.9**	730.5620	**-0.3**	730.5613	**-1.2**	730.5621	**-0.1**	730.5607	**-2.1**	730.5614	**-1.1**
DGTS(34:6)	728.5465	728.5445	**-2.7**	728.5474	**1.2**	728.5456	**-1.2**	728.5451	**-1.9**	728.5454	**-1.5**	728.5457	**-1.1**	728.5429	**-4.9**
DGTS(34:7((OH))	742.5258	-	-	-	-	742.5256	**-0.3**	-	-	742.5288	**4.0**	-	-	742.5266	**1.1**
DGTS(34:7)	726.5309	726.5283	**-3.5**	726.5335	**3.6**	726.5305	**-0.5**	726.5302	**-0.9**	726.5305	**-0.5**	726.5303	**-0.8**	726.5303	**-0.8**
DGTS(34:8)	724.5152	-	-	-	-	724.5151	**-0.1**	724.5133	**-2.6**	724.5151	**-0.1**	-	-	724.5147	**-0.7**
DGTS(35:1)	752.6398	752.6408	**1.3**	752.6410	**1.5**	-	-	752.6386	**-1.6**	-	-	752.6403	**0.6**	752.6400	**0.2**
DGTS(35:2)	750.6242	750.6250	**1.1**	-	-	750.6221	**-2.8**	750.6244	**0.3**	-	-	750.6247	**0.7**	750.6249	**0.9**
DGTS(35:3)	748.6086	748.6077	**-1.2**	748.6076	**-1.3**	748.6074	**-1.6**	748.6074	**-1.6**	-	-	748.6063	**-3.0**	-	-
DGTS(35:4)	746.5929	746.5918	**-1.5**	746.5922	**-0.9**	746.5929	**0.0**	746.5914	**-2.0**	746.5929	**0.0**	746.5915	**-1.9**	746.5915	**-1.9**
DGTS(35:5)	744.5773	744.5760	**-1.7**	744.5774	**0.2**	744.5763	**-1.3**	744.5771	**-0.2**	744.5753	**-2.6**	744.5767	**-0.8**	-	-
DGTS(35:6)	742.5616	742.5603	**-1.8**	742.5619	**0.4**	742.5607	**-1.3**	742.5620	**0.5**	742.5599	**-2.3**	-	-	-	-
DGTS(36:0)	768.6717	-	-	-	-	-	-	-	-	-	-	768.6706	**-1.4**	-	-

DGTS(36:10)	748.5152	-	-	-	-	748.5136	**-2.1**	-	-	748.5138	**-1.9**	-	-	748.5129	**-3.1**
DGTS(36:2)	764.6404	764.6411	**0.9**	764.6419	**2.0**	764.6408	**0.5**	764.6403	**-0.1**	764.6403	**-0.1**	764.6402	**-0.3**	764.6391	**-1.7**
DGTS(36:3)	762.6248	762.6227	**-2.8**	762.6212	**-4.7**	762.6210	**-5.0**	762.6212	**-4.7**	762.6219	**-3.8**	762.6247	**-0.1**	-	-
DGTS(36:4)	760.6091	760.6080	**-1.4**	760.6076	**-2.0**	760.6085	**-0.8**	760.6076	**-2.0**	760.6078	**-1.7**	760.6073	**-2.4**	760.6073	**-2.4**
DGTS(36:5)	758.5935	758.5923	**-1.6**	758.5958	**3.0**	758.5928	**-0.9**	758.5923	**-1.6**	758.5925	**-1.3**	758.5907	**-3.7**	758.5924	**-1.5**
DGTS(36:6)	756.5778	756.5759	**-2.5**	756.5780	**0.3**	756.5770	**-1.1**	756.5777	**-0.1**	756.5748	**-4.0**	756.5776	**-0.3**	756.5762	**-2.1**
DGTS(36:7)	754.5622	754.5598	**-3.2**	754.5645	**3.0**	754.5616	**-0.8**	754.5616	**-0.8**	754.5612	**-1.3**	754.5622	**0.0**	754.5607	**-2.0**
DGTS(36:8)	752.5465	752.5452	**-1.7**	-	-	752.5460	**-0.7**	752.5446	**-2.5**	752.5460	**-0.7**	752.5469	**0.5**	752.5446	**-2.5**
DGTS(36:9)	750.5309	-	-	-	-	750.5282	**-3.6**	750.5291	**-2.4**	750.5280	**-3.9**	-	-	-	-
DGTS(37:4)	774.6242	774.6237	**-0.7**	774.6260	**2.3**	774.6229	**-1.7**	774.6213	**-3.8**	-	-	774.6206	**-4.7**	-	-
DGTS(37:5)	772.6086	772.6092	**0.8**	772.6101	**2.0**	772.6086	**0.0**	772.6077	**-1.1**	-	-	772.6069	**-2.2**	-	-
DGTS(37:6)	770.5930	-	-	770.5952	**2.9**	770.5915	**-1.9**	770.5922	**-1.0**	770.5912	**-2.3**	770.5925	**-0.6**	-	-
DGTS(37:7)	768.5773	-	-	-	-	768.5765	**-1.1**	768.5780	**0.9**	768.5743	**-3.9**	768.5736	**-4.8**	-	-
DGTS(38:10)	776.5465	-	-	-	-	776.5432	**-4.2**	776.5451	**-1.8**	776.5440	**-3.2**	776.5440	**-3.2**	-	-
DGTS(38:5)	786.6248	786.6228	**-2.5**	786.6263	**1.9**	786.6228	**-2.5**	786.6222	**-3.3**	786.6235	**-1.7**	786.6216	**-4.1**	786.6225	**-2.9**
DGTS(38:6)	784.6091	784.6080	**-1.4**	784.6108	**2.2**	784.6067	**-3.1**	784.6081	**-1.3**	784.6058	**-4.2**	784.6090	**-0.1**	784.6078	**-1.7**
DGTS(38:7)	782.5935	782.5908	**-3.5**	782.5911	**-3.1**	782.5928	**-0.9**	782.5927	**-1.0**	782.5904	**-4.0**	782.5929	**-0.8**	782.5921	**-1.8**
DGTS(38:8)	780.5778	780.5741	**-4.7**	-	-	780.5756	**-2.8**	780.5760	**-2.3**	780.5741	**-4.7**	780.5743	**-4.5**	780.5743	**-4.5**
DGTS(38:9)	778.5622	-	-	-	-	778.5593	**-3.7**	778.5600	**-2.8**	-	-	778.5592	**-3.9**	778.5588	**-4.4**
DGTS(40:10)	804.5778	804.5744	**-4.2**	804.5782	**0.5**	804.5782	**0.5**	804.5778	**0.0**	804.5751	**-3.4**	804.5785	**0.9**	804.5775	**-0.4**
DGTS(40:9)	806.5935	806.5895	**-5.0**	806.5925	**-1.2**	806.5908	**-3.3**	806.5906	**-3.6**	806.5901	**-4.2**	806.5910	**-3.1**	806.5908	**-3.3**
LPC(14:0)	468.3090	468.3091	**0.2**	468.3091	**0.2**	468.3089	**-0.2**	468.3090	**0.0**	468.3090	**0.0**	468.3090	**0.0**	468.3088	**-0.4**
LPC(14:1)	466.2934	-	-	-	-	-	-	466.2933	**-0.1**	-	-	466.2933	**-0.1**	-	-
LPC(15:0)	482.3241	-	-	482.3247	**1.2**	482.3246	**1.0**	482.3246	**1.0**	482.3247	**1.2**	482.3246	**1.0**	-	-
LPC(16:0)	496.3403	496.3401	**-0.4**	496.3405	**0.4**	496.3405	**0.4**	496.3408	**1.0**	496.3406	**0.6**	496.3405	**0.4**	496.3397	**-1.2**
LPC(16:1)	494.3247	494.3237	**-2.0**	494.3250	**0.7**	494.3244	**-0.5**	494.3245	**-0.3**	494.3245	**-0.3**	494.3245	**-0.3**	494.3242	**-0.9**
LPC(16:2)	492.3090	-	-	492.3089	**-0.2**	492.3089	**-0.2**	492.3076	**-2.8**	-	-	492.3088	**-0.4**	-	-
LPC(16:3)	490.2934	-	-	490.2933	**-0.1**	490.2923	**-2.2**	490.2928	**-1.2**	490.2934	**0.1**	490.2930	**-0.7**	490.2930	**-0.7**
LPC(16:4)	488.2777	-	-	-	-	-	-	-	-	488.2775	**-0.5**	488.2774	**-0.7**	488.2771	**-1.3**
LPC(17:0)	510.3560	510.3564	**0.8**	510.3563	**0.6**	510.3568	**1.6**	510.3567	**1.4**	510.3561	**0.2**	510.3562	**0.4**	510.3559	**-0.2**
LPC(17:1)	508.3403	-	-	508.3404	**0.2**	508.3404	**0.2**	508.3403	**0.0**	508.3395	**-1.6**	508.3403	**0.0**	-	-
LPC(18:0)	524.3716	524.3717	**0.2**	524.3723	**1.3**	524.3725	**1.7**	-	-	524.3727	**2.1**	524.3724	**1.5**	524.3733	**3.2**
LPC(18:1)	522.3560	522.3561	**0.3**	522.3567	**1.4**	522.3561	**0.3**	522.3563	**0.6**	522.3565	**1.0**	522.3566	**1.2**	522.3556	**-0.7**
LPC(18:2)	520.3403	-	-	520.3408	**0.9**	520.3403	**0.0**	520.3404	**0.2**	520.3384	**-3.7**	520.3403	**0.0**	520.3402	**-0.2**
LPC(18:3)	518.3247	-	-	518.3246	**-0.1**	518.3229	**-3.4**	518.3237	**-1.9**	518.3245	**-0.3**	518.3240	**-1.3**	518.3238	**-1.7**
LPC(18:4)	516.3090	-	-	516.3074	**-3.1**	516.3068	**-4.3**	516.3068	**-4.3**	516.3090	**0.0**	516.3072	**-3.5**	516.3085	**-1.0**
LPC(18:5)	514.2934	-	-	514.2915	**-3.7**	514.2911	**-4.5**	514.2911	**-4.5**	-	-	514.2915	**-3.7**	-	-
LPC(20:0)	552.4029	-	-	552.4034	**0.9**	552.4028	**-0.2**	552.4031	**0.4**	-	-	552.4029	**0.0**	552.4006	**-4.2**

LPC(20:1)	550.3873	-	-	550.3876	**0.5**	550.3875	**0.4**	-	-	-	-	550.3876	**0.5**	550.3868	**-0.9**
LPC(20:3)	546.3560	-	-	546.3560	**0.0**	546.3567	**1.3**	546.3566	**1.1**	-	-	546.3561	**0.2**	-	-
LPC(20:4)	544.3403	544.3384	**-3.5**	544.3396	**-1.3**	544.3392	**-2.1**	544.3397	**-1.1**	544.3384	**-3.5**	544.3401	**-0.4**	544.3387	**-3.0**
LPC(20:5)	542.3247	-	-	542.3228	**-3.5**	542.3237	**-1.8**	542.3240	**-1.3**	542.3242	**-0.9**	542.3243	**-0.7**	542.3242	**-0.9**
LPC(22:0)	580.4342	-	-	-	-	-	-	-	-	-	-	580.4343	**0.1**	-	-
LPC(22:1)	578.4186	-	-	-	-	-	-	-	-	-	-	578.4191	**0.9**	-	-
LPC(22:3)	574.3873	-	-	-	-	-	-	-	-	-	-	574.3868	**-0.8**	-	-
LPC(22:4)	572.3716	-	-	572.3697	**-3.3**	-	-	-	-	-	-	572.3723	**1.2**	-	-
LPC(22:5)	570.3560	-	-	570.3559	**-0.2**	-	-	-	-	-	-	570.3556	**-0.7**	570.3553	**-1.2**
LPC(22:6)	568.3403	-	-	568.3407	**0.7**	568.3398	**-0.9**	568.3377	**-4.6**	-	-	568.3400	**-0.5**	-	-
LPE(14:0)	426.2621	-	-	426.2620	**-0.2**	426.2618	**-0.6**	426.2617	**-0.9**	426.2618	**-0.6**	426.2619	**-0.4**	-	-
LPE(15:0)	440.2777	-	-	440.2777	**0.0**	440.2774	**-0.7**	440.2776	**-0.3**	440.2778	**0.2**	440.2777	**0.0**	-	-
LPE(16:0)	454.2934	-	-	454.2935	**0.3**	454.2935	**0.3**	454.2937	**0.7**	454.2937	**0.7**	454.2936	**0.5**	454.2931	**-0.6**
LPE(16:1)	452.2777	-	-	452.2777	**0.0**	452.2778	**0.2**	452.2777	**0.0**	452.2777	**0.0**	452.2777	**0.0**	452.2775	**-0.5**
LPE(16:2)	450.2621	-	-	450.2619	**-0.4**	-	-	450.2607	**-3.0**	450.2617	**-0.8**	450.2617	**-0.8**	-	-
LPE(16:3)	448.2464	-	-	448.2460	**-0.9**	448.2447	**-3.8**	448.2443	**-4.7**	448.2452	**-2.7**	448.2446	**-4.1**	-	-
LPE(16:4)	446.2308	-	-	-	-	446.2305	**-0.6**	-	-	-	-	-	-	-	-
LPE(17:0)	468.3084	-	-	468.3096	**2.5**	-	-	468.3092	**1.6**	468.3092	**1.6**	468.3093	**1.8**	-	-
LPE(17:1)	466.2934	-	-	466.2932	**-0.4**	-	-	466.2935	**0.3**	466.2930	**-0.8**	466.2933	**-0.1**	-	-
LPE(18:1)	480.3090	-	-	480.3092	**0.4**	480.3094	**0.8**	480.3093	**0.6**	480.3092	**0.4**	480.3092	**0.4**	480.3091	**0.2**
LPE(18:2)	478.2934	-	-	478.2935	**0.3**	478.2947	**2.8**	478.2935	**0.3**	478.2941	**1.5**	478.2935	**0.3**	478.2930	**-0.8**
LPE(18:3)	476.2777	-	-	476.2768	**-1.9**	476.2771	**-1.3**	476.2768	**-1.9**	476.2771	**-1.3**	476.2766	**-2.3**	-	-
LPE(18:4)	474.2621	-	-	474.2597	**-5.0**	474.2620	**-0.1**	474.2597	**-5.0**	474.2599	**-4.6**	474.2599	**-4.6**	-	-
LPE(20:1)	508.3403	-	-	-	-	-	-	-	-	-	-	508.3399	**-0.8**	508.3396	**-1.4**
LPE(20:3)	504.3090	-	-	-	-	-	-	504.3073	**-3.4**	-	-	504.3077	**-2.6**	-	-
LPE(20:4)	502.2934	-	-	502.2914	**-3.9**	502.2917	**-3.3**	502.2934	**0.1**	502.2914	**-3.9**	502.2924	**-1.9**	502.2917	**-3.3**
LPE(20:5)	500.2777	-	-	500.2755	**-4.4**	500.2778	**0.2**	500.2776	**-0.2**	500.2762	**-3.0**	500.2769	**-1.6**	500.2771	**-1.2**
LPE(22:0)	538.3873	-	-	-	-	-	-	-	-	-	-	538.3873	**0.1**	-	-
LPG(16:0)	483.2723	483.2732	**1.9**	483.2736	**2.7**	-	-	483.2734	**2.3**	-	-	483.2738	**3.1**	-	-
LPG(18:2)	507.2723	507.2731	**1.6**	-	-	-	-	-	-	-	-	-	-	-	-
LPG(18:3)	505.2566	-	-	505.2584	**3.5**	505.2579	**2.5**	-	-	505.2580	**2.7**	-	-	-	-
MGDG(30:0)	720.5625	-	-	-	-	720.5589	**-5.0**	720.5598	**-3.7**	-	-	720.5604	**-2.9**	-	-
MGDG(30:1)	718.5464	-	-	-	-	718.5475	**1.5**	718.5469	**0.7**	-	-	718.5460	**-0.6**	-	-
MGDG(30:2)	716.5313	-	-	-	-	-	-	716.5313	**0.0**	-	-	716.5295	**-2.5**	-	-
MGDG(30:4)	712.5000	-	-	-	-	-	-	-	-	-	-	712.4992	**-1.1**	-	-
MGDG(32:0)	748.5939	748.5915	**-3.2**	-	-	-	-	-	-	-	-	-	-	-	-
MGDG(32:1)	746.5777	746.5750	**-3.6**	-	-	746.5770	**-0.9**	746.5779	**0.3**	-	-	746.5775	**-0.3**	-	-
MGDG(32:2)	744.5626	744.5615	**-1.5**	744.5610	**-2.1**	744.5626	**0.0**	744.5620	**-0.8**	-	-	744.5623	**-0.4**	744.5605	**-2.8**

MGDG(32:3)	742.5464	742.5450	**-1.9**	742.5464	**0.0**	742.5466	**0.3**	742.5466	**0.3**	742.5467	**0.4**	742.5467	**0.4**	742.5430	**-4.6**
MGDG(32:4)	740.5307	-	-	740.5303	**-0.5**	740.5300	**-0.9**	-	-	740.5319	**1.6**	740.5303	**-0.5**	740.5302	**-0.7**
MGDG(32:5)	738.5156	-	-	738.5155	**-0.1**	738.5149	**-0.9**	738.5155	**-0.1**	738.5155	**-0.1**	738.5157	**0.1**	738.5151	**-0.7**
MGDG(32:6)	736.4994	-	-	736.5001	**1.0**	736.4966	**-3.8**	736.5002	**1.1**	736.5003	**1.2**	736.5003	**1.2**	736.4994	**0.0**
MGDG(32:7)	734.4843	-	-	-	-	734.4831	**-1.6**	-	-	734.4840	**-0.4**	734.4845	**0.3**	734.4836	**-1.0**
MGDG(32:8)	732.4687	-	-	-	-	732.4673	**-1.9**	-	-	-	-	732.4692	**0.7**	-	-
MGDG(33:3)	756.5620	756.5622	**0.3**	756.5631	**1.4**	-	-	-	-	-	-	756.5612	**-1.1**	-	-
MGDG(33:4)	754.5464	-	-	754.5477	**1.7**	-	-	-	-	-	-	754.5461	**-0.4**	754.5489	**3.3**
MGDG(33:6)	750.5151	-	-	750.5159	**1.1**	-	-	-	-	-	-	-	-	750.5141	**-1.3**
MGDG(34:1)	774.6090	-	-	774.6062	**-3.6**	774.6107	**2.2**	774.6082	**-1.0**	774.6070	**-2.6**	774.6062	**-3.6**	774.6086	**-0.5**
MGDG(34:2)	772.5933	772.5907	**-3.4**	772.5930	**-0.4**	772.5940	**0.9**	772.5934	**0.1**	772.5918	**-1.9**	772.5938	**0.6**	772.5929	**-0.5**
MGDG(34:3)	770.5782	770.5777	**-0.7**	770.5742	**-5.2**	770.5776	**-0.8**	770.5786	**0.5**	770.5760	**-2.9**	770.5774	**-1.1**	770.5767	**-2.0**
MGDG(34:4)	768.5626	768.5625	**-0.1**	768.5622	**-0.5**	768.5593	**-4.3**	-	-	768.5625	**-0.1**	768.5619	**-0.9**	768.5615	**-1.4**
MGDG(34:5)	766.5469	-	-	766.5450	**-2.5**	766.5469	**0.0**	766.5465	**-0.5**	766.5477	**1.0**	766.5463	**-0.8**	766.5465	**-0.5**
MGDG(34:6)	764.5312	-	-	764.5315	**0.4**	-	-	764.5321	**1.2**	-	-	764.5311	**-0.1**	-	-
MGDG(34:7)	762.5156	-	-	-	-	762.5158	**0.3**	762.5161	**0.7**	762.5158	**0.3**	762.5152	**-0.5**	762.5153	**-0.4**
MGDG(34:8)	760.5000	-	-	-	-	760.5033	**4.3**	-	-	760.5003	**0.4**	760.4994	**-0.8**	760.4994	**-0.8**
MGDG(34:9)	758.4843	-	-	-	-	-	-	-	-	-	-	758.4873	**3.9**	758.4839	**-0.6**
MGDG(35:1)	788.6252	788.6223	**-3.6**	788.6218	**-4.3**	-	-	-	-	788.6238	**-1.7**	-	-	-	-
MGDG(35:7)	776.5307	-	-	-	-	776.5320	**1.7**	-	-	776.5315	**1.0**	-	-	776.5323	**2.1**
MGDG(36:1)	802.6403	-	-	-	-	-	-	-	-	802.6397	**-0.7**	-	-	-	-
MGDG(36:2)	800.6252	800.6236	**-2.0**	800.6223	**-3.6**	-	-	-	-	800.6229	**-2.8**	800.6262	**1.3**	-	-
MGDG(36:4)	796.5933	796.5932	**-0.1**	796.5938	**0.6**	796.5901	**-4.0**	-	-	796.5902	**-3.9**	796.5939	**0.8**	796.5900	**-4.1**
MGDG(36:5)	794.5782	794.5782	**0.0**	794.5763	**-2.4**	794.5778	**-0.5**	794.5779	**-0.4**	-	-	794.5777	**-0.6**	794.5783	**0.1**
MGDG(36:6)	792.5625	-	-	792.5627	**0.3**	792.5617	**-1.0**	792.5621	**-0.5**	792.5625	**0.0**	792.5612	**-1.6**	792.5610	**-1.9**
MGDG(36:7)	790.5469	-	-	-	-	790.5473	**0.5**	790.5463	**-0.8**	790.5472	**0.4**	790.5450	**-2.4**	790.5450	**-2.4**
MGDG(36:8)	788.5313	-	-	-	-	788.5311	**-0.3**	788.5337	**3.0**	788.5333	**2.5**	788.5299	**-1.8**	788.5291	**-2.8**
MGDG(36:9)	786.5156	-	-	-	-	-	-	-	-	-	-	786.5160	**0.5**	786.5147	**-1.1**
MGDG(38:6)	820.5939	-	-	-	-	820.5977	**4.6**	820.5927	**-1.5**	-	-	820.5972	**4.0**	820.5953	**1.7**
MGDG(38:7)	818.5782	-	-	-	-	818.5772	**-1.2**	818.5781	**-0.1**	-	-	818.5766	**-2.0**	-	-
MGDG(38:8)	816.5626	-	-	-	-	-	-	816.5618	**-1.0**	-	-	816.5607	**-2.3**	816.5593	**-4.0**
MGDG(40:10)	840.5626	-	-	-	-	840.5629	**0.4**	840.5622	**-0.5**	-	-	840.5623	**-0.4**	-	-
MGDG(40:8)	844.5939	-	-	-	-	844.5919	**-2.4**	844.5925	**-1.7**	-	-	-	-	-	-
MGMG(14:0)	482.3329	-	-	-	-	482.3325	**-0.8**	482.3327	**-0.4**	-	-	-	-	-	-
MGMG(16:0)	510.3642	510.3643	**0.2**	-	-	510.3639	**-0.6**	510.3643	**0.2**	510.3641	**-0.2**	510.3646	**0.8**	510.3652	**2.0**
MGMG(16:1)	508.3486	508.3485	**-0.1**	-	-	508.3484	**-0.3**	508.3493	**1.5**	-	-	508.3488	**0.5**	-	-
MGMG(16:2)	506.3329	506.3328	**-0.2**	506.3335	**1.2**	-	-	-	-	-	-	506.3334	**1.0**	-	-
MGMG(16:3)	504.3173	-	-	504.3171	**-0.4**	504.3173	**0.0**	-	-	-	-	504.3173	**0.0**	-	-

MGMG(16:4(OH))	518.2965	-	-	-	-	518.2965	**0.0**	-	-	-	-	-	-	-	-
MGMG(16:4)	502.3016	-	-	-	-	502.3013	**-0.6**	-	-	502.3015	**-0.2**	502.3013	**-0.6**	502.3011	**-1.0**
MGMG(18:1)	536.3799	536.3803	**0.8**	-	-	536.3797	**-0.3**	-	-	-	-	-	-	536.3791	**-1.4**
MGMG(18:2)	534.3642	534.3658	**3.0**	534.3647	**0.9**	534.3656	**2.6**	534.3661	**3.5**	534.3651	**1.7**	534.3641	**-0.2**	534.3647	**0.9**
MGMG(18:3)	532.3486	532.3485	**-0.1**	532.3488	**0.5**	532.3486	**0.1**	532.3494	**1.6**	532.3486	**0.1**	532.3487	**0.3**	532.3485	**-0.1**
MGMG(18:4)	530.3329	-	-	-	-	530.3331	**0.4**	-	-	530.3328	**-0.2**	530.3328	**-0.2**	530.3328	**-0.2**
MGMG(20:4)	558.3642	-	-	-	-	558.3632	**-1.8**	558.3653	**2.0**	-	-	-	-	-	-
MGMG(20:5)	556.3486	-	-	-	-	556.3485	**-0.2**	556.3481	**-0.9**	-	-	556.3485	**-0.2**	556.3480	**-1.1**
MGTA(16:0)	474.3795	-	-	-	-	-	-	-	-	-	-	474.3798	**0.6**	474.3789	**-1.3**
MGTA(16:1)	472.3638	-	-	-	-	-	-	-	-	-	-	472.3636	**-0.5**	472.3631	**-1.5**
MGTA(18:1)	500.3951	-	-	-	-	-	-	-	-	-	-	500.3963	**2.4**	500.3949	**-0.4**
MGTA(18:2)	498.3795	-	-	-	-	-	-	-	-	-	-	498.3795	**0.1**	-	-
MGTA(18:3)	496.3638	-	-	-	-	-	-	-	-	-	-	496.3634	**-0.8**	496.3613	**-5.1**
MGTA(20:4)	522.3795	-	-	-	-	-	-	-	-	-	-	522.3801	**1.2**	522.3787	**-1.5**
MGTA(20:5)	520.3638	-	-	-	-	-	-	-	-	-	-	520.3630	**-1.6**	520.3632	**-1.2**
MGTA(22:6)	546.3795	-	-	-	-	-	-	-	-	-	-	546.3791	**-0.7**	-	-
MGTS(14:0)	446.3482	446.3482	**0.1**	446.3480	**-0.4**	446.3481	**-0.1**	446.3481	**-0.1**	446.3481	**-0.1**	446.3481	**-0.1**	446.3479	**-0.6**
MGTS(14:1)	444.3325	-	-	-	-	444.3329	**0.9**	-	-	-	-	-	-	-	-
MGTS(15:0)	460.3633	460.3637	**0.8**	460.3638	**1.1**	460.3640	**1.5**	460.3638	**1.1**	460.3638	**1.1**	460.3638	**1.1**	460.3634	**0.2**
MGTS(16:0)	474.3795	474.3797	**0.4**	474.3795	**0.0**	474.3794	**-0.2**	474.3797	**0.4**	474.3795	**0.0**	474.3797	**0.4**	474.3793	**-0.4**
MGTS(16:1)	472.3638	472.3639	**0.2**	472.3638	**0.0**	472.3637	**-0.2**	472.3639	**0.2**	472.3638	**0.0**	472.3639	**0.2**	472.3639	**0.2**
MGTS(16:2)	470.3482	470.3501	**4.0**	-	-	470.3495	**2.8**	470.3496	**3.0**	-	-	-	-	-	-
MGTS(16:3)	468.3325	468.3307	**-3.8**	468.3302	**-4.9**	468.3334	**1.9**	468.3304	**-4.5**	468.3318	**-1.5**	468.3305	**-4.3**	468.3300	**-5.3**
MGTS(16:4)	466.3169	-	-	-	-	466.3169	**0.0**	-	-	466.3169	**0.0**	-	-	-	-
MGTS(17:0)	488.3940	488.3952	**2.4**	488.3954	**2.8**	488.3951	**2.2**	488.3948	**1.6**	488.3950	**2.0**	-	-	-	-
MGTS(17:1)	486.3789	486.3794	**1.0**	486.3794	**1.0**	486.3795	**1.2**	486.3792	**0.6**	486.3795	**1.2**	486.3809	**4.1**	-	-
MGTS(17:2)	484.3638	484.3644	**1.2**	-	-	484.3637	**-0.2**	484.3642	**0.8**	-	-	-	-	-	-
MGTS(18:0)	502.4108	502.4116	**1.6**	502.4101	**-1.4**	502.4112	**0.8**	502.4112	**0.8**	502.4121	**2.6**	-	-	-	-
MGTS(18:1)	500.3951	500.3956	**1.0**	500.3954	**0.6**	500.3950	**-0.2**	500.3955	**0.8**	500.3952	**0.2**	500.3954	**0.6**	500.3953	**0.4**
MGTS(18:2)	498.3795	498.3798	**0.7**	498.3799	**0.9**	498.3799	**0.9**	498.3801	**1.3**	498.3804	**1.9**	498.3799	**0.9**	-	-
MGTS(18:3)	496.3638	496.3624	**-2.8**	496.3629	**-1.8**	496.3631	**-1.4**	496.3619	**-3.9**	496.3628	**-2.0**	496.3621	**-3.5**	496.3615	**-4.7**
MGTS(18:4)	494.3482	494.3458	**-4.9**	494.3463	**-3.8**	494.3479	**-0.6**	494.3459	**-4.7**	494.3482	**0.0**	494.3460	**-4.5**	494.3459	**-4.7**
MGTS(18:5)	492.3325	492.3307	**-3.7**	-	-	492.3321	**-0.8**	492.3305	**-4.1**	-	-	-	-	-	-
MGTS(19:1)	514.4102	514.4107	**0.9**	514.4106	**0.7**	514.4109	**1.3**	-	-	514.4102	**0.0**	-	-	-	-
MGTS(20:0)	530.4421	-	-	-	-	530.4423	**0.4**	-	-	-	-	-	-	-	-
MGTS(20:4)	522.3795	522.3784	**-2.0**	522.3784	**-2.0**	522.3780	**-2.8**	522.3803	**1.6**	522.3772	**-4.3**	522.3777	**-3.4**	522.3772	**-4.3**
MGTS(20:5)	520.3638	520.3620	**-3.5**	520.3625	**-2.5**	520.3642	**0.8**	520.3640	**0.4**	520.3632	**-1.2**	520.3640	**0.4**	520.3636	**-0.4**

PC(28:1)	676.4917	-	-	-	-	676.4920	**0.4**	676.4919	**0.3**	-	-	676.4933	**2.4**	-	-
PC(30:0)	706.5387	706.5394	**1.0**	706.5388	**0.2**	706.5372	**-2.1**	706.5353	**-4.8**	706.5391	**0.6**	706.5395	**1.2**	706.5359	**-3.9**
PC(30:1)	704.5230	-	-	704.5254	**3.4**	704.5234	**0.5**	704.5234	**0.5**	704.5237	**1.0**	704.5238	**1.1**	-	-
PC(30:3)	700.4917	700.4896	**-3.0**	700.4899	**-2.6**	700.4895	**-3.2**	700.4898	**-2.8**	700.4894	**-3.3**	700.4896	**-3.0**	700.4897	**-2.9**
PC(30:4)	698.4761	-	-	698.4753	**-1.1**	698.4742	**-2.7**	698.4739	**-3.1**	698.4750	**-1.5**	698.4750	**-1.5**	698.4795	**4.9**
PC(31:1)	718.5387	718.5391	**0.6**	718.5382	**-0.7**	718.5388	**0.2**	718.5385	**-0.3**	718.5383	**-0.5**	718.5386	**-0.1**	718.5374	**-1.8**
PC(31:2)	716.5230	-	-	716.5221	**-1.3**	716.5220	**-1.4**	716.5223	**-1.0**	716.5216	**-2.0**	716.5233	**0.4**	716.5203	**-3.8**
PC(31:3)	714.5074	-	-	714.5075	**0.2**	714.5067	**-1.0**	714.5068	**-0.8**	714.5058	**-2.2**	714.5069	**-0.7**	714.5055	**-2.6**
PC(32:0)	734.5700	734.5699	**-0.1**	734.5684	**-2.2**	-	-	-	-	734.5664	**-4.9**	734.5669	**-4.2**	734.5665	**-4.7**
PC(32:1)	732.5543	732.5520	**-3.2**	732.5532	**-1.5**	732.5539	**-0.6**	732.5539	**-0.6**	732.5526	**-2.4**	732.5539	**-0.6**	732.5536	**-1.0**
PC(32:2)	730.5387	730.5394	**1.0**	730.5383	**-0.5**	730.5389	**0.3**	730.5389	**0.3**	730.5390	**0.4**	730.5388	**0.2**	730.5383	**-0.5**
PC(32:3)	728.5230	-	-	728.5232	**0.2**	728.5231	**0.1**	728.5234	**0.5**	728.5231	**0.1**	728.5232	**0.2**	728.5234	**0.5**
PC(32:4)	726.5074	-	-	726.5074	**0.0**	726.5062	**-1.6**	726.5065	**-1.2**	726.5072	**-0.3**	726.5072	**-0.3**	726.5074	**0.0**
PC(32:5)	724.4917	-	-	724.4916	**-0.2**	724.4910	**-1.0**	724.4909	**-1.1**	724.4917	**0.0**	724.4914	**-0.5**	724.4925	**1.1**
PC(32:6)	722.4761	-	-	722.4764	**0.4**	722.4747	**-1.9**	722.4761	**0.0**	722.4755	**-0.8**	722.4761	**0.0**	722.4762	**0.2**
PC(32:7)	720.4604	-	-	720.4573	**-4.3**	-	-	-	-	720.4617	**1.8**	720.4639	**4.8**	720.4602	**-0.3**
PC(33:0)	748.5856	748.5858	**0.2**	748.5824	**-4.3**	-	-	-	-	-	-	748.5830	**-3.5**	-	-
PC(33:2)	744.5543	744.5544	**0.1**	744.5538	**-0.7**	744.5541	**-0.3**	744.5546	**0.4**	744.5539	**-0.6**	744.5541	**-0.3**	744.5534	**-1.3**
PC(33:3)	742.5387	-	-	742.5386	**-0.1**	742.5386	**-0.1**	742.5387	**0.0**	742.5380	**-0.9**	742.5386	**-0.1**	742.5375	**-1.6**
PC(33:4)	740.5230	-	-	740.5226	**-0.6**	740.5227	**-0.4**	740.5222	**-1.1**	740.5221	**-1.3**	740.5217	**-1.8**	740.5228	**-0.3**
PC(33:5)	738.5074	-	-	738.5068	**-0.8**	738.5069	**-0.7**	-	-	738.5056	**-2.4**	738.5065	**-1.2**	738.5067	**-0.9**
PC(33:6)	736.4917	-	-	736.4916	**-0.2**	-	-	-	-	736.4914	**-0.5**	736.4914	**-0.5**	736.4934	**2.3**
PC(34:1)	760.5856	760.5834	**-2.9**	760.5828	**-3.7**	760.5839	**-2.3**	760.5840	**-2.1**	760.5824	**-4.2**	760.5842	**-1.9**	760.5850	**-0.8**
PC(34:2)	758.5700	758.5706	**0.8**	758.5691	**-1.2**	758.5695	**-0.6**	758.5699	**-0.1**	758.5696	**-0.5**	758.5692	**-1.0**	758.5691	**-1.2**
PC(34:3)	756.5543	756.5549	**0.8**	756.5541	**-0.3**	756.5546	**0.4**	756.5545	**0.2**	756.5541	**-0.3**	756.5544	**0.1**	756.5536	**-1.0**
PC(34:4)	754.5387	754.5358	**-3.8**	754.5379	**-1.0**	754.5370	**-2.2**	754.5371	**-2.1**	754.5382	**-0.6**	754.5377	**-1.3**	754.5384	**-0.4**
PC(34:5(OH))	768.5179	-	-	768.5165	**-1.8**	768.5161	**-2.3**	-	-	768.5164	**-2.0**	768.5178	**-0.1**	768.5161	**-2.3**
PC(34:5)	752.5230	-	-	752.5221	**-1.2**	752.5210	**-2.7**	752.5209	**-2.8**	752.5220	**-1.4**	752.5217	**-1.8**	752.5228	**-0.3**
PC(34:6)	750.5074	-	-	750.5074	**0.0**	750.5055	**-2.5**	750.5059	**-2.0**	750.5067	**-0.9**	750.5061	**-1.7**	750.5065	**-1.2**
PC(34:7)	748.4917	-	-	748.4896	**-2.8**	748.4886	**-4.2**	748.4887	**-4.1**	748.4914	**-0.4**	748.4901	**-2.2**	748.4908	**-1.2**
PC(34:8)	746.4761	-	-	746.4735	**-3.5**	-	-	-	-	746.4752	**-1.2**	746.4741	**-2.7**	746.4750	**-1.4**
PC(34:9)	744.4604	-	-	744.4592	**-1.7**	-	-	-	-	744.4601	**-0.4**	744.4600	**-0.6**	744.4597	**-1.0**
PC(35:1)	774.6013	774.6012	**-0.1**	774.5994	**-2.5**	774.5995	**-2.3**	774.5991	**-2.8**	774.5996	**-2.2**	774.5978	**-4.5**	774.5987	**-3.4**
PC(35:2)	772.5856	772.5860	**0.5**	772.5848	**-1.1**	772.5850	**-0.8**	772.5848	**-1.1**	772.5848	**-1.1**	772.5836	**-2.6**	772.5838	**-2.4**
PC(35:3)	770.5700	-	-	770.5695	**-0.6**	770.5693	**-0.9**	770.5695	**-0.6**	770.5664	**-4.6**	770.5679	**-2.7**	770.5686	**-1.8**
PC(35:4)	768.5543	-	-	768.5535	**-1.1**	768.5527	**-2.1**	768.5528	**-2.0**	768.5528	**-2.0**	768.5524	**-2.5**	768.5526	**-2.3**
PC(35:5)	766.5387	766.5378	**-1.2**	766.5371	**-2.1**	766.5365	**-2.8**	766.5370	**-2.2**	766.5361	**-3.4**	766.5374	**-1.7**	766.5369	**-2.3**
PC(35:6)	764.5230	-	-	764.5219	**-1.5**	-	-	764.5218	**-1.6**	764.5222	**-1.1**	764.5217	**-1.7**	764.5204	**-3.4**

PC(35:7)	762.5074	-	-	762.5048	**-3.4**	-	-	-	-	762.5088	**1.9**	762.5068	**-0.8**	762.5069	**-0.6**
PC(36:1)	788.6169	788.6149	**-2.6**	788.6132	**-4.7**	788.6160	**-1.2**	788.6148	**-2.7**	-	-	788.6132	**-4.7**	788.6138	**-4.0**
PC(36:10)	770.4761	-	-	-	-	-	-	-	-	770.4736	**-3.2**	770.4739	**-2.8**	770.4745	**-2.1**
PC(36:2)	786.6013	786.6014	**0.2**	786.6010	**-0.4**	786.6007	**-0.7**	786.6003	**-1.2**	786.6010	**-0.4**	786.6011	**-0.2**	786.6004	**-1.1**
PC(36:3)	784.5856	784.5857	**0.1**	784.5843	**-1.7**	784.5851	**-0.7**	784.5847	**-1.2**	784.5835	**-2.7**	784.5847	**-1.2**	784.5828	**-3.6**
PC(36:4(OH))	798.5649	-	-	798.5639	**-1.2**	798.5642	**-0.9**	798.5645	**-0.5**	798.5623	**-3.3**	798.5632	**-2.1**	798.5635	**-1.7**
PC(36:4)	782.5700	782.5687	**-1.6**	782.5689	**-1.4**	782.5681	**-2.4**	782.5681	**-2.4**	782.5685	**-1.9**	782.5684	**-2.0**	782.5684	**-2.0**
PC(36:5)	780.5543	780.5532	**-1.4**	780.5530	**-1.7**	780.5527	**-2.1**	780.5527	**-2.1**	780.5520	**-3.0**	780.5532	**-1.4**	780.5538	**-0.7**
PC(36:6)	778.5387	778.5392	**0.7**	778.5383	**-0.5**	778.5373	**-1.8**	778.5379	**-1.0**	778.5383	**-0.5**	778.5382	**-0.6**	778.5372	**-1.9**
PC(36:7)	776.5230	-	-	776.5200	**-3.9**	776.5207	**-3.0**	776.5209	**-2.7**	776.5221	**-1.2**	776.5225	**-0.7**	776.5220	**-1.3**
PC(36:8)	774.5074	-	-	774.5041	**-4.2**	774.5056	**-2.3**	774.5084	**1.3**	774.5052	**-2.8**	774.5056	**-2.3**	774.5058	**-2.0**
PC(36:9)	772.4917	-	-	772.4888	**-3.8**	-	-	-	-	772.4883	**-4.4**	772.4896	**-2.8**	772.4904	**-1.7**
PC(37:2)	800.6169	800.6174	**0.6**	800.6154	**-1.9**	800.6163	**-0.8**	800.6162	**-0.9**	800.6159	**-1.3**	800.6154	**-1.9**	800.6166	**-0.4**
PC(37:3)	798.6013	798.6014	**0.1**	798.6002	**-1.4**	798.5997	**-2.0**	798.5994	**-2.4**	798.5993	**-2.5**	798.5998	**-1.9**	798.5993	**-2.5**
PC(37:4)	796.5856	796.5842	**-1.8**	796.5849	**-0.9**	796.5830	**-3.3**	796.5834	**-2.8**	796.5823	**-4.2**	796.5827	**-3.7**	796.5830	**-3.3**
PC(37:5)	794.5700	794.5671	**-3.6**	794.5675	**-3.1**	794.5674	**-3.2**	794.5679	**-2.6**	794.5663	**-4.6**	794.5684	**-2.0**	794.5677	**-2.9**
PC(37:6)	792.5543	-	-	792.5512	**-3.9**	792.5523	**-2.5**	792.5529	**-1.8**	792.5504	**-4.9**	792.5520	**-2.9**	792.5518	**-3.2**
PC(37:7)	790.5387	-	-	790.5347	**-5.1**	790.5360	**-3.4**	790.5363	**-3.0**	790.5351	**-4.6**	790.5377	**-1.3**	790.5360	**-3.4**
PC(38:1)	816.6482	816.6458	**-3.0**	-	-	816.6463	**-2.4**	816.6462	**-2.5**	-	-	816.6470	**-1.5**	-	-
PC(38:10)	798.5074	-	-	-	-	798.5046	**-3.5**	798.5044	**-3.7**	798.5045	**-3.6**	798.5044	**-3.7**	798.5046	**-3.5**
PC(38:2)	814.6326	814.6327	**0.1**	814.6311	**-1.8**	814.6308	**-2.2**	814.6314	**-1.5**	-	-	814.6327	**0.1**	814.6312	**-1.7**
PC(38:3)	812.6169	-	-	812.6131	**-4.7**	812.6160	**-1.1**	812.6153	**-2.0**	812.6163	**-0.8**	812.6138	**-3.9**	812.6131	**-4.7**
PC(38:4)	810.6013	810.5990	**-2.8**	810.5985	**-3.4**	810.5973	**-4.9**	810.5978	**-4.3**	810.5986	**-3.3**	810.5984	**-3.6**	810.5982	**-3.8**
PC(38:5)	808.5856	808.5839	**-2.1**	808.5825	**-3.9**	808.5838	**-2.3**	808.5841	**-1.9**	808.5836	**-2.5**	808.5837	**-2.4**	808.5836	**-2.5**
PC(38:6)	806.5700	806.5706	**0.8**	806.5665	**-4.3**	806.5687	**-1.6**	806.5689	**-1.3**	806.5664	**-4.4**	806.5683	**-2.1**	806.5692	**-1.0**
PC(38:7)	804.5543	804.5533	**-1.3**	804.5510	**-4.1**	804.5519	**-3.0**	804.5519	**-3.0**	804.5511	**-4.0**	804.5536	**-0.9**	804.5507	**-4.5**
PC(38:8)	802.5387	-	-	802.5348	**-4.8**	802.5358	**-3.6**	802.5352	**-4.3**	802.5347	**-5.0**	802.5365	**-2.7**	802.5366	**-2.6**
PC(38:9)	800.5230	-	-	800.5204	**-3.3**	800.5205	**-3.2**	800.5197	**-4.2**	800.5195	**-4.4**	800.5195	**-4.4**	800.5218	**-1.5**
PC(40:10)	826.5387	-	-	826.5348	**-4.7**	826.5361	**-3.1**	826.5357	**-3.6**	-	-	826.5371	**-1.9**	826.5366	**-2.5**
PC(40:11)	824.5230	-	-	824.5199	**-3.8**	824.5208	**-2.7**	824.5202	**-3.4**	824.5213	**-2.1**	824.5192	**-4.6**	824.5202	**-3.4**
PC(40:2)	842.6639	-	-	842.6630	**-1.0**	-	-	-	-	-	-	842.6641	**0.3**	-	-
PC(40:3)	840.6482	-	-	840.6485	**0.3**	-	-	-	-	-	-	840.6479	**-0.4**	-	-
PC(40:4)	838.6326	838.6288	**-4.5**	838.6289	**-4.4**	838.6304	**-2.6**	838.6313	**-1.5**	-	-	838.6297	**-3.4**	-	-
PC(40:5)	836.6169	836.6156	**-1.6**	836.6153	**-2.0**	836.6138	**-3.7**	836.6162	**-0.9**	836.6164	**-0.6**	836.6131	**-4.6**	-	-
PC(40:6)	834.6013	-	-	834.6003	**-1.2**	834.5991	**-2.6**	834.5974	**-4.7**	-	-	834.5999	**-1.7**	834.6013	**0.0**
PC(40:7)	832.5856	-	-	832.5823	**-4.0**	832.5834	**-2.7**	832.5819	**-4.5**	-	-	832.5829	**-3.3**	832.5819	**-4.5**
PC(40:8)	830.5700	830.5697	**-0.3**	830.5670	**-3.6**	830.5670	**-3.6**	830.5671	**-3.5**	-	-	830.5681	**-2.3**	830.5660	**-4.8**
PC(40:9)	828.5543	-	-	828.5513	**-3.7**	828.5522	**-2.6**	828.5520	**-2.8**	-	-	828.5508	**-4.3**	828.5516	**-3.3**

PE(29:1)	648.4604	-	-	648.4607	**0.4**	-	-	-	-	648.4612	**1.2**	648.4606	**0.3**	648.4598	**-1.0**
PE(30:0)	664.4917	664.4916	**-0.2**	664.4920	**0.4**	664.4924	**1.0**	664.4927	**1.5**	664.4919	**0.3**	664.4916	**-0.2**	664.4910	**-1.1**
PE(30:1)	662.4761	-	-	662.4764	**0.5**	662.4771	**1.5**	662.4774	**2.0**	662.4762	**0.2**	662.4762	**0.2**	662.4762	**0.2**
PE(30:2)	660.4604	-	-	660.4588	**-2.5**	-	-	-	-	660.4596	**-1.3**	660.4592	**-1.9**	-	-
PE(30:3)	658.4448	658.4428	**-3.0**	658.4426	**-3.3**	658.4431	**-2.6**	658.4428	**-3.0**	658.4429	**-2.9**	658.4428	**-3.0**	658.4423	**-3.8**
PE(31:1)	676.4917	676.4925	**1.1**	676.4920	**0.4**	676.4912	**-0.8**	676.4932	**2.2**	676.4925	**1.1**	676.4922	**0.7**	676.4922	**0.7**
PE(31:2)	674.4761	-	-	674.4764	**0.5**	674.4751	**-1.5**	-	-	674.4768	**1.1**	674.4761	**0.0**	674.4758	**-0.4**
PE(31:3)	672.4604	-	-	672.4601	**-0.5**	-	-	-	-	672.4601	**-0.5**	672.4603	**-0.2**	-	-
PE(32:1)	690.5074	690.5064	**-1.4**	690.5075	**0.2**	690.5075	**0.2**	690.5075	**0.2**	690.5071	**-0.4**	690.5075	**0.2**	690.5072	**-0.3**
PE(32:2)	688.4917	688.4913	**-0.6**	688.4919	**0.2**	688.4933	**2.3**	688.4926	**1.3**	688.4922	**0.7**	688.4919	**0.2**	688.4917	**0.0**
PE(32:3)	686.4761	-	-	686.4761	**0.0**	-	-	-	-	686.4760	**-0.1**	686.4755	**-0.8**	686.4791	**4.4**
PE(32:4)	684.4604	-	-	684.4600	**-0.6**	684.4611	**1.0**	-	-	684.4592	**-1.8**	684.4586	**-2.7**	684.4579	**-3.7**
PE(32:5)	682.4448	-	-	682.4452	**0.6**	-	-	-	-	682.4432	**-2.3**	682.4425	**-3.3**	-	-
PE(33:1)	704.5230	-	-	704.5235	**0.7**	-	-	-	-	704.5223	**-1.0**	704.5242	**1.7**	-	-
PE(33:3)	700.4917	-	-	700.4918	**0.1**	-	-	-	-	700.4913	**-0.6**	700.4915	**-0.3**	700.4885	**-4.6**
PE(33:4)	698.4761	-	-	698.4746	**-2.1**	-	-	-	-	698.4742	**-2.7**	698.4744	**-2.4**	698.4738	**-3.3**
PE(33:5)	696.4604	-	-	696.4588	**-2.3**	-	-	-	-	696.4595	**-1.3**	696.4590	**-2.1**	696.4569	**-5.1**
PE(34:1)	718.5387	-	-	718.5358	**-4.0**	718.5354	**-4.6**	718.5365	**-3.0**	718.5365	**-3.0**	718.5379	**-1.1**	718.5354	**-4.6**
PE(34:2)	716.5230	716.5221	**-1.3**	716.5229	**-0.2**	716.5218	**-1.7**	716.5229	**-0.2**	716.5233	**0.4**	716.5229	**-0.2**	716.5222	**-1.1**
PE(34:3)	714.5074	714.5066	**-1.1**	714.5075	**0.2**	714.5065	**-1.2**	714.5074	**0.0**	714.5073	**-0.1**	714.5071	**-0.4**	714.5066	**-1.1**
PE(34:4)	712.4917	712.4915	**-0.3**	712.4905	**-1.7**	712.4915	**-0.3**	712.4900	**-2.4**	712.4902	**-2.2**	712.4900	**-2.4**	712.4898	**-2.7**
PE(34:5)	710.4761	710.4760	**-0.1**	710.4751	**-1.4**	710.4761	**0.0**	710.4746	**-2.1**	710.4743	**-2.5**	710.4745	**-2.2**	710.4746	**-2.1**
PE(34:6)	708.4604	-	-	708.4598	**-0.9**	-	-	-	-	708.4587	**-2.4**	708.4587	**-2.4**	708.4580	**-3.4**
PE(34:7)	706.4448	-	-	706.4435	**-1.8**	706.4431	**-2.4**	-	-	706.4427	**-2.9**	706.4420	**-3.9**	-	-
PE(35:1)	732.5543	-	-	732.5520	**-3.2**	-	-	-	-	-	-	732.5521	**-3.0**	-	-
PE(35:2)	730.5387	-	-	730.5384	**-0.4**	-	-	-	-	-	-	730.5385	**-0.2**	730.5380	**-0.9**
PE(35:3)	728.5230	-	-	728.5228	**-0.3**	-	-	-	-	-	-	728.5221	**-1.3**	-	-
PE(35:4)	726.5074	-	-	726.5057	**-2.3**	-	-	-	-	-	-	726.5048	**-3.6**	-	-
PE(35:5)	724.4917	-	-	724.4897	**-2.8**	-	-	-	-	-	-	724.4913	**-0.6**	724.4909	**-1.1**
PE(35:6)	722.4761	-	-	722.4745	**-2.2**	-	-	722.4747	**-1.9**	722.4741	**-2.7**	722.4742	**-2.6**	722.4756	**-0.7**
PE(36:2)	744.5543	744.5510	**-4.5**	744.5542	**-0.2**	744.5552	**1.2**	744.5539	**-0.6**	744.5547	**0.5**	744.5541	**-0.3**	744.5538	**-0.7**
PE(36:3)	742.5387	742.5356	**-4.2**	742.5370	**-2.3**	-	-	742.5368	**-2.5**	742.5357	**-4.0**	742.5388	**0.2**	742.5356	**-4.2**
PE(36:4)	740.5230	740.5232	**0.2**	740.5223	**-1.0**	740.5216	**-1.9**	740.5223	**-1.0**	740.5216	**-1.9**	740.5214	**-2.2**	740.5233	**0.4**
PE(36:5)	738.5074	738.5096	**3.0**	738.5066	**-1.1**	738.5079	**0.7**	738.5074	**0.0**	738.5064	**-1.3**	738.5063	**-1.5**	738.5091	**2.3**
PE(36:6)	736.4917	736.4919	**0.2**	736.4910	**-1.0**	736.4900	**-2.4**	736.4919	**0.2**	736.4915	**-0.3**	736.4918	**0.1**	736.4920	**0.4**
PE(36:7)	734.4761	-	-	734.4729	**-4.3**	734.4744	**-2.3**	734.4738	**-3.1**	734.4733	**-3.8**	734.4743	**-2.4**	734.4729	**-4.3**
PE(37:3)	756.5543	-	-	756.5535	**-1.1**	-	-	-	-	-	-	756.5545	**0.2**	-	-
PE(37:4)	754.5387	-	-	754.5376	**-1.4**	-	-	-	-	-	-	754.5370	**-2.2**	-	-

PE(37:5)	752.5230	752.5222	**-1.1**	752.5212	**-2.4**	-	-	-	-	-	-	752.5213	**-2.3**	-	-
PE(38:2)	772.5856	-	-	-	-	-	-	-	-	-	-	772.5855	**-0.2**	-	-
PE(38:3)	770.5700	-	-	-	-	-	-	-	-	-	-	770.5695	**-0.6**	-	-
PE(38:4)	768.5543	-	-	-	-	-	-	-	-	-	-	768.5519	**-3.2**	-	-
PE(38:5)	766.5387	766.5397	**1.3**	-	-	-	-	766.5384	**-0.4**	-	-	766.5362	**-3.2**	766.5392	**0.7**
PE(38:6)	764.5230	-	-	-	-	-	-	764.5226	**-0.6**	-	-	764.5222	**-1.1**	764.5235	**0.6**
PE(38:7)	762.5077	-	-	762.5042	**-4.5**	762.5110	**4.4**	762.5068	**-1.1**	-	-	762.5067	**-1.2**	-	-
PE(38:8)	760.4917	760.4893	**-3.2**	760.4884	**-4.4**	760.4907	**-1.4**	760.4892	**-3.3**	760.4884	**-4.4**	760.4890	**-3.6**	-	-
PE(38:9)	758.4761	-	-	758.4725	**-4.7**	758.4733	**-3.7**	758.4739	**-2.9**	758.4728	**-4.3**	758.4737	**-3.1**	-	-
PE(40:10)	784.4917	784.4955	**4.8**	-	-	784.4929	**1.5**	784.4915	**-0.3**	-	-	784.4913	**-0.6**	784.4957	**5.1**
PE(40:6)	792.5543	-	-	-	-	-	-	-	-	-	-	792.5544	**0.1**	792.5539	**-0.5**
PE(40:7)	790.5387	790.5379	**-1.0**	-	-	-	-	790.5348	**-4.9**	-	-	790.5368	**-2.4**	790.5398	**1.4**
PE(40:8)	788.5230	788.5219	**-1.4**	-	-	788.5221	**-1.2**	788.5227	**-0.4**	-	-	788.5216	**-1.8**	788.5207	**-3.0**
PE(40:9)	786.5074	-	-	-	-	786.5070	**-0.5**	786.5071	**-0.4**	-	-	786.5073	**-0.1**	786.5063	**-1.4**
PG(30:0)	693.4707	693.4719	**1.7**	693.4719	**1.7**	693.4722	**2.2**	693.4718	**1.6**	693.4718	**1.6**	693.4719	**1.7**	-	-
PG(30:1)	691.4550	-	-	691.4561	**1.6**	691.4561	**1.6**	691.4557	**1.0**	691.4562	**1.7**	691.4557	**1.0**	-	-
PG(31:0)	707.4863	707.4862	**-0.1**	707.4871	**1.1**	707.4876	**1.8**	707.4869	**0.8**	707.4863	**0.0**	707.4871	**1.1**	-	-
PG(31:1)	705.4707	-	-	705.4716	**1.3**	705.4731	**3.4**	705.4718	**1.6**	705.4718	**1.6**	705.4718	**1.6**	-	-
PG(32:0)	721.5020	721.5031	**1.5**	721.5029	**1.2**	721.5034	**1.9**	721.4985	**-4.9**	721.5016	**-0.6**	721.4988	**-4.4**	721.5018	**-0.3**
PG(32:1)	719.4863	719.4870	**1.0**	719.4873	**1.4**	719.4875	**1.7**	719.4872	**1.3**	719.4870	**1.0**	719.4870	**1.0**	719.4867	**0.6**
PG(32:2(OH))	733.4656	-	-	-	-	733.4667	**1.5**	-	-	733.4671	**2.0**	-	-	-	-
PG(32:2)	717.4707	717.4734	**3.8**	717.4717	**1.4**	717.4726	**2.6**	717.4720	**1.8**	717.4714	**1.0**	717.4715	**1.1**	717.4727	**2.8**
PG(33:0)	735.5181	-	-	735.5183	**0.2**	735.5168	**-1.8**	735.5156	**-3.4**	735.5169	**-1.7**	735.5146	**-4.8**	735.5172	**-1.3**
PG(33:1)	733.5025	733.5037	**1.6**	733.5029	**0.5**	733.5028	**0.4**	733.5033	**1.1**	733.5019	**-0.8**	733.5019	**-0.8**	733.5019	**-0.8**
PG(33:2)	731.4869	731.4884	**2.1**	731.4869	**0.0**	731.4876	**1.0**	731.4895	**3.6**	731.4862	**-0.9**	731.4862	**-0.9**	731.4858	**-1.5**
PG(33:3)	729.4713	-	-	729.4716	**0.5**	729.4718	**0.7**	-	-	729.4706	**-0.9**	-	-	729.4698	**-2.0**
PG(34:0)	749.5332	-	-	749.5307	**-3.4**	-	-	749.5298	**-4.6**	-	-	-	-	-	-
PG(34:1(OH))	763.5125	763.5103	**-2.9**	-	-	763.5126	**0.1**	-	-	763.5117	**-1.0**	-	-	763.5133	**1.0**
PG(34:1)	747.5176	747.5141	**-4.7**	747.5170	**-0.8**	747.5190	**1.8**	747.5170	**-0.8**	747.5180	**0.5**	747.5183	**0.9**	747.5182	**0.8**
PG(34:2(OH))	761.4969	761.4971	**0.3**	-	-	761.4982	**1.7**	761.4973	**0.5**	761.4976	**0.9**	-	-	-	-
PG(34:2)	745.5020	745.5030	**1.3**	745.5022	**0.3**	745.5023	**0.4**	745.5030	**1.3**	745.5026	**0.8**	745.5026	**0.8**	-	-
PG(34:3(OH))	759.4812	759.4814	**0.3**	759.4807	**-0.7**	759.4827	**2.0**	-	-	759.4793	**-2.5**	-	-	759.4811	**-0.1**
PG(34:3)	743.4863	743.4877	**1.9**	743.4872	**1.2**	743.4880	**2.3**	743.4874	**1.5**	743.4853	**-1.3**	743.4871	**1.1**	743.4871	**1.1**
PG(34:4(OH))	757.4656	-	-	757.4658	**0.3**	757.4663	**0.9**	-	-	757.4665	**1.2**	-	-	757.4630	**-3.4**
PG(34:4)	741.4707	741.4706	**-0.1**	741.4719	**1.6**	741.4720	**1.8**	741.4691	**-2.2**	741.4720	**1.8**	741.4710	**0.4**	741.4715	**1.1**
PG(34:5(OH))	755.4499	-	-	-	-	755.4510	**1.5**	-	-	755.4505	**0.8**	-	-	-	-
PG(34:5)	739.4550	-	-	739.4561	**1.5**	739.4554	**0.5**	739.4559	**1.2**	739.4554	**0.5**	-	-	-	-
PG(34:6)	737.4393	-	-	737.4406	**1.8**	-	-	737.4429	**4.9**	-	-	-	-	-	-

PG(35:1)	761.5338	761.5326	**-1.6**	761.5325	**-1.7**	-	-	761.5369	**4.1**	761.5309	**-3.8**	761.5333	**-0.7**	-	-
PG(35:2)	759.5182	759.5181	**-0.1**	759.5176	**-0.8**	-	-	-	-	759.5176	**-0.8**	-	-	-	-
PG(35:3)	757.5019	757.5015	**-0.6**	757.5022	**0.3**	757.5026	**0.9**	-	-	757.4998	**-2.8**	-	-	757.5022	**0.3**
PG(35:4)	755.4863	-	-	755.4863	**0.0**	-	-	-	-	755.4864	**0.1**	-	-	-	-
PG(35:5)	753.4707	-	-	-	-	753.4677	**-4.0**	753.4712	**0.7**	-	-	-	-	-	-
PG(36:0)	777.5645	777.5636	**-1.2**	777.5643	**-0.3**	-	-	-	-	-	-	-	-	-	-
PG(36:1)	775.5489	775.5484	**-0.6**	775.5474	**-1.9**	775.5455	**-4.4**	775.5503	**1.8**	775.5464	**-3.2**	-	-	775.5459	**-3.9**
PG(36:2)	773.5333	773.5334	**0.1**	773.5333	**0.0**	773.5347	**1.8**	773.5343	**1.3**	773.5340	**0.9**	773.5337	**0.5**	773.5333	**0.0**
PG(36:3)	771.5176	771.5176	**0.0**	771.5181	**0.6**	771.5151	**-3.2**	-	-	771.5169	**-0.9**	771.5211	**4.5**	771.5170	**-0.8**
PG(36:4)	769.5020	769.5030	**1.3**	769.5034	**1.8**	-	-	-	-	769.5022	**0.3**	-	-	-	-
PG(36:5(OH))	783.4812	-	-	-	-	783.4827	**1.9**	783.4819	**0.9**	-	-	-	-	783.4808	**-0.5**
PG(36:5)	767.4863	-	-	767.4865	**0.3**	767.4872	**1.2**	767.4867	**0.5**	767.4866	**0.4**	767.4836	**-3.5**	-	-
PG(36:6(OH))	781.4656	-	-	-	-	781.4675	**2.4**	781.4678	**2.8**	-	-	781.4660	**0.5**	-	-
PG(36:6)	765.4707	-	-	765.4722	**2.0**	765.4722	**2.0**	765.4737	**3.9**	765.4742	**4.6**	765.4722	**2.0**	-	-
PG(38:2)	801.5646	-	-	-	-	801.5665	**2.4**	-	-	801.5642	**-0.5**	801.5653	**0.9**	-	-
PG(40:2)	829.5959	-	-	-	-	-	-	-	-	829.5969	**1.2**	-	-	-	-
PG(40:3)	827.5802	-	-	-	-	-	-	-	-	827.5817	**1.8**	-	-	-	-
PI(30:1)	779.4711	-	-	-	-	-	-	779.4711	**0.0**	-	-	-	-	-	-
PI(32:1)	807.5024	-	-	-	-	807.5019	**-0.6**	807.5003	**-2.6**	-	-	807.5019	**-0.6**	-	**#REF!**
PI(32:2)	805.4867	805.4882	**1.9**	-	-	-	-	805.4858	**-1.1**	-	-	-	-	-	-
PI(32:3)	803.4716	-	-	803.4702	**-1.8**	-	-	-	-	-	-	-	-	-	-
PI(34:1)	835.5339	835.5374	**4.2**	835.5304	**-4.2**	835.5341	**0.2**	835.5298	**-4.9**	835.5339	**0.0**	835.5297	**-5.0**	835.5343	**0.5**
PI(34:2)	833.5182	-	-	833.5166	**-1.9**	833.5154	**-3.4**	833.5148	**-4.1**	833.5147	**-4.2**	-	-	833.5158	**-2.9**
PI(34:3)	831.5025	-	-	831.5056	**3.7**	831.4994	**-3.7**	831.5013	**-1.4**	831.4998	**-3.2**	-	-	831.5002	**-2.8**
PI(34:5)	827.4711	-	-	827.4730	**2.3**	-	-	-	-	-	-	-	-	-	-
PI(36:5)	855.5024	-	-	855.5060	**4.2**	855.5036	**1.4**	855.4998	**-3.0**	855.5033	**1.1**	855.5010	**-1.6**	855.5037	**1.5**
PI-Cer(d18:1/14:0)	750.4921	-	-	-	-	750.4937	**2.1**	750.4931	**1.3**	-	-	750.4925	**0.5**	-	-
PI-Cer(d18:1/14:1)	748.4765	-	-	-	-	748.4780	**2.0**	748.4774	**1.2**	-	-	-	-	-	-
PI-Cer(t18:0/16:0)	796.5340	796.5333	**-0.8**	796.5311	**-3.6**	-	-	-	-	-	-	-	-	-	-
PI-Cer(t18:0/18:0)	824.5653	824.5656	**0.4**	824.5670	**2.1**	-	-	-	-	-	-	-	-	-	-
SQDG(28:0)	737.4510	-	-	-	-	737.4513	**0.4**	737.4509	**-0.1**	-	-	737.4517	**0.9**	-	-
SQDG(30:0)	765.4823	765.4840	**2.3**	765.4805	**-2.3**	765.4825	**0.3**	765.4820	**-0.4**	765.4817	**-0.8**	765.4820	**-0.4**	765.4827	**0.6**
SQDG(30:1)	763.4666	-	-	-	-	763.4678	**1.6**	763.4675	**1.2**	763.4668	**0.3**	763.4672	**0.8**	763.4662	**-0.5**
SQDG(30:2)	761.4510	-	-	-	-	-	-	761.4528	**2.4**	-	-	761.4518	**1.1**	-	-
SQDG(30:3)	759.4353	-	-	-	-	-	-	-	-	-	-	759.4355	**0.2**	-	-
SQDG(30:4)	757.4197	-	-	-	-	-	-	-	-	-	-	757.4199	**0.3**	-	-
SQDG(31:1)	777.4823	-	-	-	-	777.4829	**0.8**	777.4831	**1.0**	-	-	777.4808	**-1.9**	-	-

SQDG(32:0)	793.5136	793.5147	**1.4**	793.5145	**1.2**	793.5145	**1.2**	-	-	793.5147	**1.4**	-	-	793.5140	**0.5**
SQDG(32:1)	791.4979	791.4989	**1.2**	791.4974	**-0.7**	791.4991	**1.5**	791.4987	**1.0**	791.4989	**1.2**	791.4984	**0.6**	791.4985	**0.7**
SQDG(32:2)	789.4823	789.4841	**2.3**	789.4841	**2.3**	789.4834	**1.4**	789.4833	**1.3**	789.4817	**-0.7**	789.4831	**1.0**	789.4828	**0.7**
SQDG(32:3)	787.4666	-	-	787.4678	**1.5**	787.4681	**1.9**	787.4679	**1.7**	787.4676	**1.3**	787.4676	**1.3**	787.4673	**0.9**
SQDG(32:4)	785.4510	-	-	785.4516	**0.8**	785.4527	**2.2**	-	-	785.4523	**1.7**	785.4517	**0.9**	785.4522	**1.5**
SQDG(34:0)	821.5449	821.5399	**-6.1**	821.5468	**2.3**	821.5455	**0.8**	-	-	-	-	-	-	-	-
SQDG(34:1)	819.5292	819.5257	**-4.3**	819.5301	**1.1**	819.5307	**1.8**	819.5303	**1.3**	819.5302	**1.2**	819.5302	**1.2**	819.5299	**0.8**
SQDG(34:2)	817.5136	817.5148	**1.5**	817.5137	**0.2**	-	-	817.5147	**1.4**	-	-	817.5141	**0.6**	-	-
SQDG(34:3)	815.4979	815.4995	**1.9**	815.4989	**1.2**	815.4990	**1.3**	815.5006	**3.3**	815.4991	**1.4**	815.4987	**0.9**	815.4985	**0.7**
SQDG(34:4)	813.4823	813.4821	**-0.2**	813.4841	**2.2**	813.4838	**1.8**	-	-	813.4833	**1.2**	813.4826	**0.4**	813.4834	**1.4**
SQDG(34:5)	811.4666	-	-	811.4689	**2.8**	811.4683	**2.1**	-	-	811.4679	**1.6**	811.4673	**0.9**	811.4660	**-0.7**
SQDG(36:0)	849.5762	849.5760	**-0.2**	-	-	849.5770	**1.0**	-	-	-	-	-	-	-	-
SQDG(36:1)	847.5605	847.5587	**-2.2**	847.5594	**-1.3**	847.5607	**0.2**	-	-	847.5612	**0.8**	847.5608	**0.3**	847.5605	**0.0**
SQDG(36:2)	845.5449	845.5460	**1.3**	845.5443	**-0.7**	845.5438	**-1.3**	-	-	845.5430	**-2.2**	845.5450	**0.1**	845.5450	**0.1**
SQDG(36:3)	843.5292	843.5294	**0.2**	843.5319	**3.2**	843.5280	**-1.5**	-	-	843.5300	**0.9**	843.5265	**-3.2**	843.5294	**0.2**
SQDG(36:4)	841.5136	841.5128	**-0.9**	841.5172	**4.3**	841.5143	**0.9**	841.5101	**-4.1**	841.5152	**1.9**	-	-	841.5126	**-1.2**
SQDG(36:5)	839.4979	-	-	839.4981	**0.2**	839.4980	**0.1**	839.4985	**0.7**	-	-	839.4982	**0.4**	839.4978	**-0.1**
SQDG(36:6)	837.4823	-	-	837.4833	**1.2**	837.4835	**1.4**	-	-	837.4826	**0.4**	837.4831	**1.0**	837.4836	**1.6**
SQDG(36:7)	835.4666	-	-	-	-	835.4681	**1.8**	-	-	835.4688	**2.6**	835.4677	**1.3**	-	-
SQDG(38:1)	875.5918	-	-	-	-	-	-	-	-	875.5924	**0.7**	875.5921	**0.3**	-	-
SQDG(38:2)	873.5762	-	-	-	-	-	-	-	-	-	-	873.5765	**0.4**	-	-
SQDG(38:5)	867.5292	-	-	-	-	-	-	-	-	-	-	867.5304	**1.4**	867.5295	**0.3**
SQDG(40:1)	903.6231	-	-	-	-	-	-	-	-	-	-	903.6238	**0.7**	-	-
SQDG(40:2)	901.6075	-	-	-	-	-	-	-	-	-	-	901.6081	**0.7**	-	-
SQMG(16:0)	555.2839	555.2850	**2.0**	-	-	555.2851	**2.1**	-	-	-	-	555.2841	**0.3**	-	-
SQMG(18:2)	579.2839	579.2841	**0.3**	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide

Table S6.2. Fatty acyl chain composition of the 109 polar lipid species common in the lipidome of *Spirulina* sp., *Chlorella vulgaris*, *Chlorococcum amblystomatis*, *Nannochloropsis oceanica*, *Scenedesmus obliquus*, *Phaeodactylum tricornutum* and *Tetraselmis chuii*.

Lipid species	**Calculated *m/z***	**Fatty acyl chains identified in MS/MS spectra**						
		***Spirulina* sp.**	***C. vulgaris***	***C. amblystomatis***	***N. oceanica***	***S. obliquus***	***P. tricornutum***	***T. chui***
DGDG(32:1)	908.6310	-	16:0-16:1	16:0-16:1	16:0-16:1	-	16:0-16:1	16:0-16:1 14:0-18:1
DGDG(32:2)	906.6154	-	-	16:1-16:1 16:0-16:2	16:1-16:1 16:0-16:2 14:0-18:2	-	16:1-16:1 16:0-16:2	16:1-16:1 16:0-16:2 14:0-18:2 17:1-15:1
DGDG(34:3)	932.6310	16:0-18:3 16:1-18:2	16:0-18:3 16:1-18:2 18:1-16:2	16:0-18:3 16:1-18:2	-	16:0-18:3 16:1-18:2 18:1-16:2	-	16:0-18:3 16:1-18:2 18:1-16:2
DGDG(34:5)	928.5997	18:3-16:2 16:1-18:4	18:3-16:2 16:3-18:2	18:3-16:2 16:3-18:2 18:1-16:4 16:1-18:4 14:0-20:5	14:0-20:5	-	-	18:3-16:2 16:3-18:2 18:1-16:4 16:1-18:4
DGDG(35:5)	942.6154	-	-	-	15:0-20:5	-	-	-
DGMG(16:0)	672.4170	16:0	16:0	16:0	16:0	-	-	16:0
DGMG(18:2)	696.4170	-	-	-	-	-	-	-
DGTS(30:1)	682.5622	16:1-14:0	16:1-14:0 15:1-15:0	16:1-14:0	16:1-14:0	16:1-14:0 15:1-15:0	16:1-14:0	-
DGTS(31:1)	696.5773	16:1-15:0 17:1-14:0	16:1-15:0 17:1-14:0	-	16:1-15:0 17:1-14:0	-	16:1-15:0	16:1-15:0
DGTS(32:1)	710.5935	16:1-16:0 18:1-14:0 17:1-15:0	16:1-16:0 18:1-14:0 17:1-15:0	16:1-16:0	16:1-16:0 18:1-14:0	16:1-16:0 18:1-14:0	16:1-16:0 18:1-14:0	16:1-16:0
DGTS(32:2)	708.5778	-	-	16:1-16:1 16:2-16:0	16:1-16:1 16:2-16:0	-	16:1-16:1	16:1-16:1

					18:2-14:0			
DGTS(32:3)	706.5622	16:1-16:2 18:3-14:0	-	16:3-16:0	-	-	-	-
DGTS(33:1)	724.6091	-	18:1-15:0 17:1-16:0	-	-	-	18:1-15:0	-
DGTS(33:2)	722.5929	17:1-16:1 18:2-15:0 16:2-17:0	-	17:1-16:1 17:2-16:0 16:2-17:0	17:1-16:1 18:2-15:0 17:2-16:0	-	18:2-15:0 15:1-18:1	-
DGTS(33:3)	720.5767	-	18:3-15:0 17:3-16:0	-	-	-	-	-
DGTS(33:4)	718.5605	-	18:4-15:0	18:4-15:0 17:4-16:0 17:3-16:1 16:4-17:0 15:1-18:3 17:2-16:2	-	18:4-15:0 17:4-16:0 17:3-16:1 16:4-17:0 15:1-18:3	-	-
DGTS(34:1)	738.6248	-	16:0-18:1 19:1-15:0	16:0-18:1	16:0-18:1 20:1-14:0	-	16:0-18:1 16:1-18:0	16:1-18:0
DGTS(34:2)	736.6091	18:1-16:1 18:2-16:0	17:1-17:1 19:2-15:0	18:1-16:1 18:2-16:0 20:2-14:0	18:1-16:1 18:2-16:0 20:2-14:0	-	18:1-16:1	-
DGTS(34:3)	734.5935	-	-	16:1-18:2 20:3-14:0 16:2-18:1	16:1-18:2 20:3-14:0 18:3-16:0	-	18:0-16:3	-
DGTS(34:4)	732.5778	16:2-18:2 18:3-16:1	18:4-16:0 18:3-16:1	18:4-16:0 18:3-16:1	20:4-14:0	18:4-16:0 18:3-16:1	16:2-18:2	16:2-18:2
DGTS(34:5)	730.5622	-	-	18:4-16:1 18:1-16:4	20:5-14:0	18:4-16:1 18:1-16:4	-	18:2-16:3

				18:2-16:3				
DGTS(34:6)	728.5465	-	-	-	20:5-14:1	-	-	-
DGTS(34:7)	726.5309	-	-	16:4-18:3 16:3-18:4	-	16:4-18:3 16:3-18:4	-	16:4-18:3 16:2-18:5
DGTS(35:4)	746.5929	15:0-20:4	-	17:0-18:4	-	17:0-18:4	-	-
DGTS(36:2)	764.6404	18:1-18:1 20:1-16:1 20:0-16:2	-	-	-	18:1-18:1	18:1-18:1	18:1-18:1
DGTS(36:4)	760.6091	18:2-18:2 18:3-18:1 20:4-16:0 20:3-16:1	18:2-18:2	18:2-18:2 18:3-18:1 20:4-16:0 20:3-16:1 20:2-16:2 20:1-16:3 20:0-16:4	-	18:2-18:2 18:3-18:1 18:1-18:0 20:3-16:1 20:0-16:4	18:3-18:1	18:0-18:4
DGTS(36:5)	758.5935	20:4-16:1 18:3-18:2	-	20:5-16:0 18:4-18:1 20:4-16:1 20:3-16:2 20:2-16:3 20:1-16:4	20:5-16:0	-	20:5-16:0	20:1-16:4
DGTS(36:6)	756.5778	-	-	16:1-20:5 20:3-16:3 18:2-18:4 18:3-18:3	16:1-20:5	-	-	-
DGTS(36:7)	754.5622	-	-	18:4-18:3	16:2-20:5	18:4-18:3	16:3-20:4	-
DGTS(38:5)	786.6248	-	20:2-18:3	-	20:4-18:1 20:5-18:0	-	18:1-20:4	20:4-18:1 20:5-18:0

DGTS(38:6)	784.6091	18:2-20:4	22:5-16:1 22:6-16:0	20:5-18:1	-	-	20:5-18:1	20:3-18:3
DGTS(38:7)	782.5935	22:6-16:1	-	20:5-18:2	20:5-18:2 18:3-20:4	-	-	-
DGTS(40:10)	804.5778	-	-	20:5-20:5	20:5-20:5	-	20:5-20:5	20:5-20:5
DGTS(40:9)	806.5935	-	-	20:4-20:5	-	-	20:4-20:5	20:4-20:5
LPC(14:0)	468.3090	-	-	-	14:0	-	14:0	-
LPC(16:0)	496.3403	16:0	16:0	-	16:0	16:0	16:0	16:0
LPC(16:1)	494.3247	-	-	16:1	16:1	-	16:1	16:1
LPC(17:0)	510.3560	-	-	-	17:0	-	17:0	-
LPC(18:1)	522.3560	18:1	-	18:1	-	18:1	18:1	18:1
LPC(20:4)	544.3403	-	-	-	20:4	20:4	20:4	20:4
MGDG(32:3)	742.5464	-	-	-	-	-	16:1-16:2 16:0-16:3	-
MGDG(34:2)	772.5933	-	16:0-18:2 16:1-18:1 17:2-17:0 17:1-17:1	16:0-18:2	16:0-18:2 16:1-18:1	16:0-18:2 16:1-18:1	-	-
MGDG(34:3)	770.5782	16:0-18:3	16:0-18:3 18:2-16:1 18:1-16:2	16:0-18:3 18:2:16:1	-	16:0-18:3 18:2-16:1 18:1-16:2	-	-
MGMG(18:2)	534.3642	18:2	18:2	-	-	-	-	-
MGMG(18:3)	532.3486	-	18:3	18:3	-	18:3	-	18:3
MGTS(14:0)	446.3482	14:0	-	14:0	14:0	14:0	-	14:0
MGTS(15:0)	460.3633	15:0	15:0	-	15:0	15:0	-	-
MGTS(16:0)	474.3795	-	16:0	16:0	-	16:0	16:0	16:0
MGTS(16:1)	472.3638	16:1	16:1	16:1	16:1	16:1	16:1	-
MGTS(16:3)	468.3325	-	-	-	16:3	-	-	-
MGTS(18:1)	500.3951	-	18:1	18:1	18:1	18:1	18:1	18:1

MGTS(18:3)	496.3638	18:3	18:3	-	18:3	18:3	-	18:3
MGTS(18:4)	494.3482	-	-	18:4	18:4	18:4	-	-
MGTS(20:4)	522.3795	20:4	20:4	-	-	-	20:4	-
MGTS(20:5)	520.3638	-	-	20:5	20:5	-	20:5	-
PC(30:0)	706.5387	-	-	-	-	-	16:0-14:0	-
PC(30:3)	700.4917	-	16:3-14:0	-	-	-	16:3-14:0	-
PC(31:1)	718.5387	-	-	-	16:1-15:0 17:1-14:0 15:1-16:0	-	16:1-15:0 17:1-14:0 15:1-16:0	16:1-15:0
PC(32:1)	732.5543	-	16:1-16:0 14:0-18:1 15:0-17:1	16:1-16:0	-	-	-	16:1-16:0 14:0-18:1
PC(32:2)	730.5387	-	-	16:1-16:1	16:1-16:1 16:2-16:0 18:2-14:0	-	16:1-16:1 16:2-16:0	16:1-16:1 16:2-16:0
PC(33:2)	744.5543	-	-	17:1-16:1	17:1-16:1 17:2-16:0 18:2-15:0	-	17:1-16:1 17:2-16:0	-
PC(34:1)	760.5856	18:1-16:0 16:1-18:0	18:1-16:0 17:0-17:1	18:1-16:0 16:1-18:0	18:1-16:0 16:1-18:0	-	18:1-16:0 16:1-18:0	18:1-16:0 16:1-18:0
PC(34:2)	758.5700	16:1-18:1 18:2-16:0	-	16:1-18:1 18:2-16:0	16:1-18:1 18:2-16:0	-	16:1-18:1 18:2-16:0 16:2-18:0 20:2-14:0	16:1-18:1 18:2-16:0
PC(34:3)	756.5543	18:2-16:1 18:3-16:0	18:2-16:1 18:3-16:0 18:1-16:2	18:2-16:1	18:2-16:1		18:2-16:1 18:3-16:0 18:1-16:2 20:3-14:0	18:2-16:1 18:3-16:0 18:1-16:2
PC(34:4)	754.5387	-	18:2-16:2	18:2-16:2	18:2-16:2		18:2-16:2	18:2-16:2

			18:3-16:1	18:3-16:1	18:3-16:1		18:3-16:1	18:3-16:1
			18:1-16:3		18:1-16:3		18:1-16:3	18:1-16:3
					18:4-16:0		18:4-16:0	18:4-16:0
					20:4-14:0		20:4-14:0	
PC(35:1)	774.6013	-	-	-	16:1-17:0	-	18:1-17:0	18:1-17:0
							17:1-18:0	19:1-16:0
PC(35:2)	772.5856	18:2-17:0	18:2-17:0	-	-	-	18:2-17:0	18:2-17:0
		18:1-17:1					18:1-17:1	18:1-17:1
		19:1-16:1						19:1-16:1
PC(35:5)	766.5387	-	-	-	-	-	15:0-20:5	-
							17:2-18:3	
PC(36:2)	786.6013	18:1-18:1	18:1-18:1	18:1-18:1	18:1-18:1	18:1-18:1	18:1-18:1	18:1-18:1
		19:1-17:1	18:2-18:0		18:2-18:0		18:2-18:0	18:2-18:0
					20:1-16:1		20:1-16:1	20:1-16:1
					20:2-16:0		20:2-16:0	
							16:2-20:0	
PC(36:3)	784.5856	-	18:2-18:1	18:2-18:1	18:2-18:1	-	18:2-18:1	-
					16:1-20:2		16:1-20:2	
					20:3-16:0			
PC(36:4)	782.5700	18:2-18:2	18:2-18:2	18:2-18:2	18:2-18:2		18:2-18:2	-
		20:4-16:0	18:3-18:1	20:4-16:0	20:4-16:0		20:4-16:0	
		19:1-17:3			20:3-16:1		20:3-16:1	
		18:3-18:1					18:3-18:1	
							20:2-16:2	
							18:4-18:0	
PC(36:5)	780.5543	-	18:2-18:3	18:2-18:3	16:0-20:5		16:0-20:5	18:2-18:3
				16:0-20:5	16:1-20:4			16:0-20:5
				16:1-20:4				16:1-20:4

								18:4-18:1 16:4-20:1
PC(36:6)	778.5387	-	18:3-18:3	16:1-20:5	16:1-20:5 16:2-20:4		16:1-20:5 16:2-20:4 18:4-18:2 18:3-18:3	16:1-20:5 18:4-18:2 18:3-18:3
PC(37:2)	800.6169	18:1-19:1	18:1-19:1	-	-	-	18:1-19:1	18:1-19:1
PC(37:3)	798.6013	-	18:2-19:1	-	-	-	18:2-19:1 18:1-19:2	-
PC(37:4)	796.5856	-	18:3-19:1 18:2-19:2	-	-	-	-	18:3-19:1
PC(37:5)	794.5700	-	19:2-18:3	-	-	-	17:1-20:4	18:4-19:1
PC(38:4)	810.6013	-	20:1-18:3 18:2-20:2	-	-	-	20:3-18:1	-
PC(38:5)	808.5856	-	20:2-18:3 18:2-20:3	20:4-18:1	20:4-18:1	-	20:4-18:1 18:0-20:5 18:2-20:3	20:4-18:1 16:0-22:5
PC(38:6)	806.5700	-	18:3-20:3 18:2-20:4	18:1-20:5 18:2-20:4	18:1-20:5 18:2-20:4 18:3-20:3	-	18:1-20:5 18:2-20:4	18:1-20:5
PC(38:7)	804.5543	-	18:3-20:4	18:2-20:5	18:2-20:5 18:3-20:4	-	18:2-20:5 18:3-20:4 16:1-22:6	-
PE(30:0)	664.4917	15:0-15:0 14:0-16:0	15:0-15:0 14:0-16:0	-	15:0-15:0 14:0-16:0	-	-	15:0-15:0
PE(30:3)	658.4448	-	-	-	-	-	-	-
PE(31:1)	676.4917	-	16:1-15:0 16:0-15:1 17:1-14:0	16:1-15:0	16:1-15:0	16:1-15:0	16:1-15:0 16:0-15:1 17:1-14:0	16:1-15:0 17:1-14:0

			18:1-13:0				18:1-13:0	
PE(32:1)	690.5074	16:0-16:1 17:1-15:0	16:0-16:1 17:1-15:0	16:0-16:1	16:0-16:1 17:1-15:0 14:0-18:1	16:0-16:1	16:0-16:1 17:1-15:0 14:0-18:1 15:1-17:0	-
PE(32:2)	688.4917	16:1-16:1 16:2-16:0 15:1-17:1 18:2-14:0	16:1-16:1 16:2-16:0	16:1-16:1	16:1-16:1 16:2-16:0	16:1-16:1	16:1-16:1 16:2-16:0 15:1-17:1 18:2-14:0 18:1-14:1	16:1-16:1 16:2-16:0
PE(34:2)	716.5230	-	16:1-18:1 18:2-16:0	16:1-18:1 18:2-16:0	16:1-18:1	16:1-18:1	16:1-18:1 18:2-16:0 17:1-17:1	16:1-18:1 18:2-16:0
PE(34:3)	714.5074	18:3-16:0 18:2-16:1	18:3-16:0 18:2-16:1 18:1-16:2	-	18:2-16:1 20:3-14:0	18:3-16:0	18:3-16:0 18:2-16:1 18:1-16:2	18:3-16:0 18:2-16:1
PE(34:4)	712.4917	-	18:3-16:1 18:2-16:2	18:4-16:0	18:3-16:1 14:0-20:4	18:3-16:1	18:3-16:1 18:2-16:2 18:4-16:0	18:3-16:1
PE(34:5)	710.4761	-	18:2-16:3 16:2-18:3	16:1-18:4	-	-	18:2-16:3 16:2-18:3 16:1-18:4 14:0-20:5	-
PE(36:2)	744.5543	18:1-18:1	18:1-18:1 18:2-18:0	18:1-18:1	18:1-18:1		18:1-18:1 18:2-18:0 20:2-16:0 16:1-20:1	18:1-18:1
PE(36:4)	740.5230	-	18:1-18:3 18:2-18:2	-	20:3-16:1 20:4-16:0	18:1-18:3	-	-

PE(36:5)	738.5074	-	18:2-18:3	18:4-18:1	16:1-20:4	18:2-18:3	18:2-18:3 16:1-20:4 16:0-20:5	-
PE(36:6)	736.4917	-	18:3-18:3	18:2-18:4	16:1-20:5 16:2-20:4	18:3-18:3	18:3-18:3 16:1-20:5 16:2-20:4	-
PG(32:0)	721.5020	-	16:0-16:0	-	-	-	-	-
PG(32:1)	719.4863	16:0-16:1	16:0-16:1 15:0-17:1	16:0-16:1 14:0-18:1	16:0-16:1 14:0-18:1	16:0-16:1	16:0-16:1 14:0-18:1	16:0-16:1 14:0-18:1
PG(32:2)	717.4707	-	16:1-16:1 16:2-16:0 18:2-14:0 18:1-14:1 17:2-15:0	16:1-16:1	16:1-16:1 16:2-16:0	16:1-16:1	16:1-16:1 16:2-16:0	-
PG(33:1)	733.5025	16:0-17:1 18:1-15:0	16:0-17:1	-	16:1-17:0	18:1-15:0	-	-
PG(33:2)	731.4869	16:0-17:2 15:0-18:2 18:1-15:2 16:1-17:1	17:0-16:2	-	-	-	-	-
PG(34:1)	747.5176	-	16:0-18:1 17:0-17:1	16:0-18:1	16:0-18:1	-	-	16:0-18:1
PG(34:3)	743.4863	16:0-18:3 16:1-18:2	16:0-18:3 16:1-18:2 17:0-17:3 17:2-17:1	16:0-18:3	16:1-18:2	-	16:0-18:3 16:1-18:2	16:0-18:3
PG(34:4)	741.4707	-	18:3-16:1 18:2-16:2 17:2-17:2	18:3-16:1 18:4-16:0	-	18:3-16:1	18:3-16:1 18:2-16:2 18:4-16:0	18:3-16:1 18:4-16:0

PG(36:2)	773.5333	18:1-18:1 18:2-18:0 16:0-20:2	18:1-18:1 18:2-18:0	18:1-18:1	18:1-18:1	18:1-18:1	18:1-18:1 18:2-18:0	18:1-18:1 18:2-18:0 16:0-20:2
PI(34:1)	835.5339			18:1-16:0	18:1-16:0 16:1-18:0		18:1-16:0 16:1-18:0	18:1-16:0
SQDG(30:0)	765.4823	16:0-14:0	-	16:0-14:0	16:0-14:0	16:0-14:0	16:0-14:0	-
SQDG(32:1)	791.4979	16:0-16:1	16:0-16:1	16:0-16:1	-	-	16:0-16:1	16:0-16:1
SQDG(32:2)	789.4823	-	-	16:1-16:1 16:0-16:2	16:1-16:1	16:1-16:1	16:1-16:1 16:0-16:2	-
SQDG(34:1)	819.5292	-	-	16:0-18:1	16:0-18:1	-	16:0-18:1	16:0-18:1
SQDG(34:3)	815.4979	18:3-16:0	18:3-16:0	18:3-16:0 18:1-16:2	-	18:2-16:1	18:3-16:0 18:2-16:1 20:3-14:0	18:3-16:0

Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide

Table S6.3. The 193 common polar lipid species observed among microalgae of the Chlorophyta phylum (*Tetraselmis chuii* (T.c), *Scenedesmus obliquus* (S.o), *Chlorococcum amblystomatis* (C.a), *Chlorella vulgaris* (C.v)) .

		Phospholipids		
LPC(14:0)	PC(31:2)	PC(35:3)	PC(38:8)	PG(32:1)
LPC(16:0)	PC(31:3)	PC(35:4)	PC(38:9)	PG(32:2)
LPC(16:1)	PC(32:1)	PC(35:5)	PC(40:11)	PG(33:0)
LPC(16:3)	PC(32:2)	PC(36:2)	PE(30:0)	PG(33:1)
LPC(17:0)	PC(32:3)	PC(36:3)	PE(30:1)	PG(33:2)
LPC(18:0)	PC(32:4)	PC(36:4(OH))	PE(30:3)	PG(33:3)
LPC(18:1)	PC(32:5)	PC(36:4)	PE(31:1)	PG(34:1)
LPC(18:2)	PC(32:6)	PC(36:5)	PE(31:2)	PG(34:3(OH))
LPC(18:3)	PC(33:2)	PC(36:6)	PE(32:1)	PG(34:3)
LPC(18:4)	PC(33:3)	PC(36:7)	PE(32:2)	PG(34:4(OH))
LPC(20:4)	PC(33:4)	PC(36:8)	PE(32:4)	PG(34:4)
LPC(20:5)	PC(33:5)	PC(37:2)	PE(34:1)	PG(35:3)
LPE(16:0)	PC(34:1)	PC(37:3)	PE(34:2)	PG(36:1)
LPE(16:1)	PC(34:2)	PC(37:4)	PE(34:3)	PG(36:2)
LPE(18:1)	PC(34:3)	PC(37:5)	PE(34:4)	PG(36:3)
LPE(18:2)	PC(34:4)	PC(37:6)	PE(34:5)	PI(34:1)
LPE(20:4)	PC(34:5(OH))	PC(37:7)	PE(36:2)	PI(34:2)
LPE(20:5)	PC(34:5)	PC(38:3)	PE(36:4)	PI(34:3)
PC(30:0)	PC(34:6)	PC(38:4)	PE(36:5)	PI(36:5)
PC(30:3)	PC(34:7)	PC(38:5)	PE(36:6)	
PC(30:4)	PC(35:1)	PC(38:6)	PE(36:7)	
PC(31:1)	PC(35:2)	PC(38:7)	PG(32:0)	
		Glycolipids		
DGDG(32:1)	DGDG(34:6)	MGDG(32:3)	MGDG(36:6)	SQDG(34:3)
DGDG(32:2)	DGDG(35:3)	MGDG(32:4)	MGMG(18:2)	SQDG(34:4)
DGDG(32:3)	DGDG(35:5)	MGDG(32:5)	MGMG(18:3)	SQDG(34:5)
DGDG(32:4)	DGDG(36:2)	MGDG(32:6)	SQDG(30:0)	SQDG(36:1)
DGDG(33:3)	DGDG(36:4)	MGDG(34:1)	SQDG(32:0)	SQDG(36:2)
DGDG(34:1)	DGDG(36:6)	MGDG(34:2)	SQDG(32:1)	SQDG(36:3)
DGDG(34:2)	DGDG(36:7)	MGDG(34:3)	SQDG(32:2)	SQDG(36:4)
DGDG(34:3)	DGMG(16:0)	MGDG(34:4)	SQDG(32:3)	SQDG(36:6)
DGDG(34:4)	DGMG(18:2)	MGDG(34:5)	SQDG(32:4)	
DGDG(34:5)	DGMG(18:3)	MGDG(36:4)	SQDG(34:1)	
		Betaine lipids		
DGTS(30:1)	DGTS(33:3)	DGTS(34:7)	DGTS(38:6)	MGTS(16:3)
DGTS(30:3)	DGTS(33:4)	DGTS(35:4)	DGTS(38:7)	MGTS(18:1)
DGTS(31:1)	DGTS(34:1)	DGTS(36:2)	DGTS(40:10)	MGTS(18:3)
DGTS(32:1)	DGTS(34:2)	DGTS(36:4)	DGTS(40:9)	MGTS(18:4)
DGTS(32:2)	DGTS(34:3)	DGTS(36:5)	MGTS(14:0)	MGTS(20:4)
DGTS(32:3)	DGTS(34:4)	DGTS(36:6)	MGTS(15:0)	MGTS(20:5)
DGTS(33:1)	DGTS(34:5)	DGTS(36:7)	MGTS(16:0)	

DGTS(33:2)
DGTS(34:6)
DGTS(38:5)
MGTS(16:1)

Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide

Table S6.4. The 264 common polar lipid species observed among microalgae of the Ochrophyta phylum (*Nannochloropsis oceanica* (N.o), *Phaeodactylum tricornutum* (P.t)).

Phospholipids

LPC(14:0)
LPC(14:1)
LPC(15:0)
LPC(16:0)
LPC(16:1)
LPC(16:2)
LPC(16:3)
LPC(17:0)
LPC(17:1)
LPC(18:1)
LPC(18:2)
LPC(18:3)
LPC(18:4)
LPC(18:5)
LPC(20:0)
LPC(20:3)
LPC(20:4)
LPC(20:5)
LPC(22:6)
LPE(14:0)
LPE(15:0)
LPE(16:0)
LPE(16:1)
LPE(16:2)
LPE(16:3)
LPE(17:0)
LPE(17:1)
LPE(18:1)
LPE(18:2)
LPE(18:3)
LPE(18:4)
LPE(20:3)
LPE(20:4)
LPE(20:5)
LPG(16:0)
PC(28:1)
PC(30:0)
PC(30:1)
PC(30:3)
PC(30:4)
PC(31:1)
PC(31:2)
PC(31:3)
PC(32:1)
PC(32:2)
PC(32:3)
PC(32:4)
PC(32:5)
PC(32:6)
PC(33:2)
PC(33:3)
PC(33:4)
PC(34:1)
PC(34:2)
PC(34:3)
PC(34:4)
PC(34:5)
PC(34:6)
PC(34:7)
PC(35:1)
PC(35:2)
PC(35:3)
PC(35:4)
PC(35:5)
PC(35:6)
PC(36:1)
PC(36:2)
PC(36:3)
PC(36:4(OH))
PC(36:4)
PC(36:5)
PC(36:6)
PC(36:7)
PC(36:8)
PC(37:2)
PC(37:3)
PC(37:4)
PC(37:5)
PC(37:6)
PC(37:7)
PC(38:1)
PC(38:10)
PC(38:2)
PC(38:3)
PC(38:4)
PC(38:5)
PC(38:6)
PC(38:7)
PC(38:8)
PC(38:9)
PC(40:10)
PC(40:11)
PC(40:4)
PC(40:5)
PC(40:6)
PC(40:7)
PC(40:8)
PC(40:9)
PE(30:0)
PE(30:1)
PE(30:3)
PE(31:1)
PE(32:1)
PE(32:2)
PE(34:1)
PE(34:2)
PE(34:3)
PE(34:4)
PE(34:5)
PE(35:6)
PE(36:2)
PE(36:3)
PE(36:4)
PE(36:5)
PE(36:6)
PE(36:7)
PE(38:5)
PE(38:6)
PE(38:7)
PE(38:8)
PE(38:9)
PE(40:10)
PE(40:7)
PE(40:8)
PE(40:9)
PG(30:0)
PG(30:1)
PG(31:0)
PG(31:1)
PG(32:0)
PG(32:1)
PG(32:2)
PG(33:0)
PG(33:1)
PG(33:2)
PG(34:1)
PG(34:2)
PG(34:3)
PG(34:4)
PG(35:1)
PG(36:2)
PG(36:5)
PG(36:6(OH))
PG(36:6)
PI(32:1)
PI(34:1)
PI(36:5)

Glycolipids

DGDG(30:1)
DGDG(30:2)
DGDG(32:1)
DGDG(32:2)
DGDG(32:3)
DGDG(32:4)
DGDG(32:5)
DGDG(34:2)
DGDG(34:3)
DGDG(34:5)
DGDG(34:6)
DGDG(35:5)
DGDG(36:5)
DGDG(36:6)
DGDG(36:7)
DGDG(38:6)
DGDG(38:7)
DGDG(40:10)
DGMG(16:0)
DGMG(16:1)
DGMG(18:2)
DGMG(20:5)
MGDG(30:0)
MGDG(30:1)
MGDG(30:2)
MGDG(32:1)
MGDG(32:2)
MGDG(32:3)
MGDG(32:5)
MGDG(32:6)
MGDG(34:1)
MGDG(34:2)
MGDG(34:3)
MGDG(34:5)
MGDG(34:6)
MGDG(34:7)
MGDG(36:5)
MGDG(36:6)
MGDG(36:7)
MGDG(36:8)
MGDG(38:6)
MGDG(38:7)
MGDG(38:8)
MGDG(40:10)
MGMG(16:0)
MGMG(16:1)
MGMG(18:2)
MGMG(18:3)
MGMG(20:5)
SQDG(28:0)
SQDG(30:0)
SQDG(30:1)
SQDG(30:2)
SQDG(31:1)
SQDG(32:1)
SQDG(32:2)
SQDG(32:3)
SQDG(34:1)
SQDG(34:2)
SQDG(34:3)
SQDG(36:5)

Betaine lipids

DGTA(40:10)
DGTS(30:1)
DGTS(30:2)
DGTS(31:1)
DGTS(32:1)
DGTS(32:2)
DGTS(32:3)
DGTS(32:4)
DGTS(33:1)
DGTS(33:2)
DGTS(33:3)
DGTS(33:4)
DGTS(34:1)
DGTS(34:2)
DGTS(34:3)
DGTS(34:4)
DGTS(34:5)
DGTS(34:6)
DGTS(34:7)
DGTS(35:1)
DGTS(35:2)
DGTS(35:3)
DGTS(35:4)
DGTS(35:5)
DGTS(36:2)
DGTS(36:3)
DGTS(36:4)
DGTS(36:5)
DGTS(36:6)
DGTS(36:7)
DGTS(36:8)
DGTS(37:4)
DGTS(37:5)
DGTS(37:6)
DGTS(37:7)
DGTS(38:10)
DGTS(38:5)
DGTS(38:6)
DGTS(38:7)
DGTS(38:8)
DGTS(38:9)
DGTS(40:10)
DGTS(40:9)
MGTS(14:0)
MGTS(15:0)
MGTS(16:0)
MGTS(16:1)
MGTS(16:3)
MGTS(17:1)
MGTS(18:1)
MGTS(18:2)
MGTS(18:3)
MGTS(18:4)
MGTS(20:4)
MGTS(20:5)

Sphingolipids

PI-Cer(d18:1/14:0)

Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide

Table S6.5. The 143 common polar lipid species observed among freshwater microalgae (*Scenedesmus obliquus* (S.o), *Chlorococcum amblystomatis* (C.a), *Chlorella vulgaris* (C.v), *Spirulina* sp.).

Phospholipids

LPC(14:0)
LPC(16:0)
LPC(16:1)
LPC(17:0)
PC(34:1)
PC(34:2)
PC(34:3)
PC(34:4)
PC(37:3)
PC(37:4)
PC(37:5)
PC(38:4)
PE(34:2)
PE(34:3)
PE(34:4)
PE(34:5)
PG(32:2)
PG(33:1)
PG(33:2)
PG(34:1)

LPC(18:0)	PC(35:1)	PC(38:5)	PE(36:2)	PG(34:2)
LPC(18:1)	PC(35:2)	PC(38:6)	PE(36:4)	PG(34:3(OH))
LPC(20:4)	PC(35:5)	PC(38:7)	PE(36:5)	PG(34:3)
PC(30:0)	PC(36:2)	PC(40:5)	PE(36:6)	PG(34:4)
PC(30:3)	PC(36:3)	PE(30:0)	PE(38:8)	PG(35:3)
PC(31:1)	PC(36:4)	PE(30:3)	PG(30:0)	PG(36:1)
PC(32:1)	PC(36:5)	PE(31:1)	PG(31:0)	PG(36:2)
PC(32:2)	PC(36:6)	PE(32:1)	PG(32:0)	PG(36:3)
PC(33:2)	PC(37:2)	PE(32:2)	PG(32:1)	PI(34:1)
		Glycolipids		
DGDG(32:1)	DGDG(35:5)	MGDG(34:2)	SQDG(32:0)	SQDG(36:2)
DGDG(32:2)	DGDG(36:2)	MGDG(34:3)	SQDG(32:1)	SQDG(36:3)
DGDG(33:3)	DGDG(36:4)	MGDG(34:4)	SQDG(32:2)	SQDG(36:4)
DGDG(34:3)	DGMG(16:0)	MGDG(36:4)	SQDG(34:1)	
DGDG(34:4)	DGMG(18:2)	MGMG(18:2)	SQDG(34:3)	
DGDG(34:5)	DGMG(18:3)	MGMG(18:3)	SQDG(34:4)	
DGDG(35:3)	MGDG(32:3)	SQDG(30:0)	SQDG(36:1)	
		Betaine lipids		
DGTS(30:1)	DGTS(33:4)	DGTS(35:6)	DGTS(40:10)	MGTS(18:1)
DGTS(30:3)	DGTS(34:1)	DGTS(36:2)	DGTS(40:9)	MGTS(18:2)
DGTS(31:1)	DGTS(34:2)	DGTS(36:3)	MGTS(14:0)	MGTS(18:3)
DGTS(31:3)	DGTS(34:3)	DGTS(36:4)	MGTS(15:0)	MGTS(18:4)
DGTS(32:1)	DGTS(34:4)	DGTS(36:5)	MGTS(16:0)	MGTS(19:1)
DGTS(32:2)	DGTS(34:5)	DGTS(36:6)	MGTS(16:1)	MGTS(20:4)
DGTS(32:3)	DGTS(34:6)	DGTS(36:7)	MGTS(16:3)	MGTS(20:5)
DGTS(33:1)	DGTS(34:7)	DGTS(38:5)	MGTS(17:0)	
DGTS(33:2)	DGTS(35:4)	DGTS(38:6)	MGTS(17:1)	
DGTS(33:3)	DGTS(35:5)	DGTS(38:7)	MGTS(18:0)	

Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide

Table S6.6. The 199 common polar lipid species observed among saline microalgae (*Tetraselmis chuii* (T.c), *Nannochloropsis oceanica* (N.o), *Phaeodactylum tricornutum* (P.t)).

		Phospholipids		
LPC(14:0)	PC(31:3)	PC(36:1)	PC(38:9)	PE(36:6)
LPC(16:0)	PC(32:1)	PC(36:2)	PC(40:10)	PE(36:7)
LPC(16:1)	PC(32:2)	PC(36:3)	PC(40:11)	PE(38:5)
LPC(16:3)	PC(32:3)	PC(36:4(OH))	PC(40:6)	PE(38:6)

LPC(17:0)
LPC(18:1)
LPC(18:2)
LPC(18:3)
LPC(18:4)
LPC(20:0)
LPC(20:4)
LPC(20:5)
LPE(16:0)
LPE(16:1)
LPE(18:1)
LPE(18:2)
LPE(20:4)
LPE(20:5)
PC(30:0)
PC(30:3)
PC(30:4)
PC(31:1)
PC(31:2)
PC(32:4)
PC(32:5)
PC(32:6)
PC(33:2)
PC(33:3)
PC(33:4)
PC(34:1)
PC(34:2)
PC(34:3)
PC(34:4)
PC(34:5)
PC(34:6)
PC(34:7)
PC(35:1)
PC(35:2)
PC(35:3)
PC(35:4)
PC(35:5)
PC(35:6)
PC(36:4)
PC(36:5)
PC(36:6)
PC(36:7)
PC(36:8)
PC(37:2)
PC(37:3)
PC(37:4)
PC(37:5)
PC(37:6)
PC(37:7)
PC(38:10)
PC(38:2)
PC(38:3)
PC(38:4)
PC(38:5)
PC(38:6)
PC(38:7)
PC(38:8)
PC(40:7)
PC(40:8)
PC(40:9)
PE(30:0)
PE(30:1)
PE(30:3)
PE(31:1)
PE(32:1)
PE(32:2)
PE(34:1)
PE(34:2)
PE(34:3)
PE(34:4)
PE(34:5)
PE(35:6)
PE(36:2)
PE(36:3)
PE(36:4)
PE(36:5)
PE(40:10)
PE(40:7)
PE(40:8)
PE(40:9)
PG(32:0)
PG(32:1)
PG(32:2)
PG(33:0)
PG(33:1)
PG(33:2)
PG(34:1)
PG(34:3)
PG(34:4)
PG(36:2)
PI(34:1)
PI(36:5)

Glycolipids

DGDG(32:1)
DGDG(32:2)
DGDG(32:3)
DGDG(32:4)
DGDG(32:5)
DGDG(34:2)
DGDG(34:3)
DGDG(34:5)
DGDG(34:6)
DGDG(35:5)
DGDG(36:5)
DGDG(36:6)
DGDG(36:7)
DGDG(38:6)
DGMG(16:0)
DGMG(18:2)
MGDG(32:2)
MGDG(32:3)
MGDG(32:5)
MGDG(32:6)
MGDG(34:1)
MGDG(34:2)
MGDG(34:3)
MGDG(34:5)
MGDG(34:7)
MGDG(36:5)
MGDG(36:6)
MGDG(36:7)
MGDG(36:8)
MGDG(38:6)
MGDG(38:8)
MGMG(16:0)
MGMG(18:2)
MGMG(18:3)
MGMG(20:5)
SQDG(30:0)
SQDG(30:1)
SQDG(32:1)
SQDG(32:2)
SQDG(32:3)
SQDG(34:1)
SQDG(34:3)
SQDG(36:5)

Betaine lipids

DGTA(40:10)
DGTS(30:1)
DGTS(31:1)
DGTS(32:1)
DGTS(32:2)
DGTS(32:3)
DGTS(32:4)
DGTS(33:1)
DGTS(33:2)
DGTS(33:3)
DGTS(33:4)
DGTS(34:1)
DGTS(34:2)
DGTS(34:3)
DGTS(34:4)
DGTS(34:5)
DGTS(34:6)
DGTS(34:7)
DGTS(35:1)
DGTS(35:2)
DGTS(35:4)
DGTS(36:2)
DGTS(36:4)
DGTS(36:5)
DGTS(36:6)
DGTS(36:7)
DGTS(36:8)
DGTS(38:5)
DGTS(38:6)
DGTS(38:7)
DGTS(38:8)
DGTS(38:9)
DGTS(40:10)
DGTS(40:9)
MGTS(14:0)
MGTS(15:0)
MGTS(16:0)
MGTS(16:1)
MGTS(16:3)
MGTS(18:1)
MGTS(18:3)
MGTS(18:4)
MGTS(20:4)
MGTS(20:5)

Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide

Table S6.7. The relative amount of the polar lipid classes observed in the lipidome of *Spirulina* sp., *Chlorella vulgaris*, *Chlorococcum amblystomatis*, *Nannochloropsis oceanica*, *Scenedesmus obliquus*, *Phaeodactylum tricornutum* and *Tetraselmis chuii*.

		Relative amount of each lipid class						
		Spirulina sp.	*C. vulgaris*	*C. amblystomatis*	*N. oceanica*	*S. obliquus*	*P. tricornutum*	*T. chuii*
Glycolipids (GL)	DGDG	24.0±1.4	30.8±4.5	83.4±13.1	53.3±8.6	21.9±1.5	25.7±2.8	53.8±3.0
	DGMG	2.6±0.3	0.1±0.0	8.0±1.4	1.1±0.2	0.4±0.1	2.2±0.5	0.5±0.1
	MGDG	90.5±9.0	113.2±15.9	93.2±14.7	66.3±9.6	94.8±5.6	114.3±13.6	162.2±14.6
	MGMG	5.2±0.7	0.6±0.1	20.4±3.6	1.1±0.2	4.2±0.4	2.5±0.4	1.7±0.5
	SQDG	177.0±18.9	32.0±3.3	125.1±23.1	83.4±12.5	63.3±13.0	138.7±24.7	105.7±11.3
	SQMG	0.4±0.1	0.0±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.3±0.1	0.0±0.0
	Total	**299.7±17.3**	**176.8±23.2**	**330.3±54.3**	**205±30.7**	**184.7±19.1**	**283.7±41.1**	**323.9±21.1**
Betaine lipids (BL)	DGTS	2.5±0.5	0.9±0.1	66.6±7.8	47.4±4.8	44.4±2.6	1.3±0.2	0.6±0.0
	MGTS	0.2±0.0	0.3±0.0	10.8±1.9	1.7±0.3	1.7±0.2	0.1±0.0	0.1±0.0
	DGTA	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.0±0.0	8.2±1.3	32.3±1.6
	MGTA	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.2±0.0
	Total	**2.7±0.5**	**1.2±0.1**	**77.4±9.7**	**49.2±5.1**	**46.1±2.8**	**9.8±1.5**	**33.2±1.6**
Phospholipids (PL)	PC	1.1±0.1	89.8±5.7	35.6±6.6	75.7±9.1	23.7±3.5	54.0±6.4	30.5±1.2
	LPC	0.0±0.0	1.6±0.1	1.3±0.2	2.3±0.4	0.4±0.1	6.2±1.1	0.5±0.1
	PE	0.5±0.2	28.5±2.1	2.3±0.4	4.6±1.4	15.1±1.3	6.5±1.0	1.5±0.7
	LPE	0.0±0.0	1.3±0.1	0.2±0.0	0.3±0.1	0.3±0.0	0.3±0.1	0.0±0.0
	PG	139.3±17.2	109.6±11.6	50.0±8.5	42.3±6.4	60.5±7.9	47.4±8.2	53.6±9.1
	LPG	1.0±0.3	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.1±0.0	0.0±0.0
	PI	0.2±0.0	3.9±0.4	13.0±2.5	12.7±2.5	1.8±0.6	10.8±2.5	5.5±0.7
	Total	**142.2±17.6**	**234.8±19.8**	**102.5±17.9**	**137.9±19.8**	**101.7±11.3**	**125.3±18.9**	**91.6±8.6**
Sphingolipids	Cer	0.0±0.0	0.5±0.1	1.0±0.2	2.2±0.3	0.5±0.2	0.0±0.0	0.0±0.0

(SL)	PI-Cer	0.5±0.1	0.5±0.1	1.3±0.2	3.3±0.7	0.0±0.0	0.0±0.0	0.0±0.0
	Total	**0.5±0.1**	**1.0±0.1**	**2.4±0.4**	**5.5±1.0**	**0.5±0.2**	**0.0±0.0**	**0.0±0.0**

Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide

Table S6.8. Results of One-way ANOVA (FDR adjusted) for statistically significant differences of polar lipid classes between microalgae.

	variables	**DFn**	**DFd**	**F**	**p**	**p<.05**	**ges**	**p.adj**	**p.adj.signif**
1	Cer	6	28	324.185	1.57E-24	*	0.986	3.73E-24	****
2	DGDG	6	28	75.452	5.55E-16	*	0.942	6.59E-16	****
3	DGMG	6	28	198.703	1.29E-21	*	0.977	2.45E-21	****
4	DGTA	6	28	4221.591	4.80E-40	*	0.999	9.12E-39	****
5	DGTS	6	28	1246.308	1.20E-32	*	0.996	1.14E-31	****
6	LPC	6	28	327.433	1.37E-24	*	0.986	3.72E-24	****
7	LPE	6	28	303.125	3.96E-24	*	0.985	8.36E-24	****
8	LPG	6	28	82.152	1.82E-16	*	0.946	2.31E-16	****
9	MGDG	6	28	19.974	6.21E-09	*	0.811	6.21E-09	****
10	MGMG	6	28	174.049	7.83E-21	*	0.974	1.14E-20	****
11	MGTA	6	28	402.31	7.98E-26	*	0.989	2.53E-25	****
12	MGTS	6	28	644.19	1.17E-28	*	0.993	5.56E-28	****
13	PC	6	28	560.754	8.04E-28	*	0.992	3.06E-27	****
14	PE	6	28	131.231	3.56E-19	*	0.966	4.83E-19	****
15	PG	6	28	36.71	5.18E-12	*	0.887	5.47E-12	****
16	PI	6	28	188.161	2.71E-21	*	0.976	4.29E-21	****
17	PI-Cer	6	28	1086.98	8.10E-32	*	0.996	5.13E-31	****
18	SQDG	6	28	52.18	6.46E-14	*	0.918	7.22E-14	****
19	SQMG	6	28	195.583	1.60E-21	*	0.977	2.76E-21	****

Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide

Table S6.9. Results of Tukey's multiple comparison test (FDR adjusted) for statistically significant differences of polar lipid classes between microalgae (*Tetraselmis chuii* (T.c), *Scenedesmus obliquus* (S.o), *Chlorococcum amblystomatis* (C.a), *Chlorella vulgaris* (C.v), *Nannochloropsis oceanica* (N.o), *Phaeodactylum tricornutum* (P.t) and *Spirulina* sp.(SP)).

	variable	**group1**	**group2**	**null.value**	**estimate**	**conf.low**	**conf.high**	**p.adj**	**p.adj.signif**
1	Cer	1.T.c	2.S.o	0	0.9972	0.8185	1.1758	0.00000	****
2	Cer	1.T.c	3.C.a	0	1.3510	1.1723	1.5297	0.00000	****
3	Cer	1.T.c	4.C.v	0	1.0564	0.8777	1.2350	0.00000	****
4	Cer	1.T.c	5.N.o	0	1.6906	1.5120	1.8693	0.00000	****
5	Cer	1.T.c	6.P.t	0	0.0000	-0.1787	0.1787	1.00000	ns
6	Cer	1.T.c	7.SP	0	0.0000	-0.1787	0.1787	1.00000	ns
7	Cer	2.S.o	3.C.a	0	0.3538	0.1752	0.5325	0.00002	****
8	Cer	2.S.o	4.C.v	0	0.0592	-0.1195	0.2378	0.93700	ns
9	Cer	2.S.o	5.N.o	0	0.6935	0.5148	0.8721	0.00000	****

10	Cer	2.S.o	6.P.t	0	-0.9972	-1.1758	-0.8185	0.00000	****
11	Cer	2.S.o	7.SP	0	-0.9972	-1.1758	-0.8185	0.00000	****
12	Cer	3.C.a	4.C.v	0	-0.2946	-0.4733	-0.1160	0.00027	***
13	Cer	3.C.a	5.N.o	0	0.3396	0.1610	0.5183	0.00003	****
14	Cer	3.C.a	6.P.t	0	-1.3510	-1.5297	-1.1723	0.00000	****
15	Cer	3.C.a	7.SP	0	-1.3510	-1.5297	-1.1723	0.00000	****
16	Cer	4.C.v	5.N.o	0	0.6343	0.4556	0.8129	0.00000	****
17	Cer	4.C.v	6.P.t	0	-1.0564	-1.2350	-0.8777	0.00000	****
18	Cer	4.C.v	7.SP	0	-1.0564	-1.2350	-0.8777	0.00000	****
19	Cer	5.N.o	6.P.t	0	-1.6906	-1.8693	-1.5120	0.00000	****
20	Cer	5.N.o	7.SP	0	-1.6906	-1.8693	-1.5120	0.00000	****
21	Cer	6.P.t	7.SP	0	0.0000	-0.1787	0.1787	1.00000	ns
22	DGDG	1.T.c	2.S.o	0	-0.3909	-0.5048	-0.2771	0.00000	****
23	DGDG	1.T.c	3.C.a	0	0.1852	0.0714	0.2991	0.00032	***
24	DGDG	1.T.c	4.C.v	0	-0.2455	-0.3594	-0.1316	0.00000	****
25	DGDG	1.T.c	5.N.o	0	-0.0090	-0.1229	0.1049	1.00000	ns
26	DGDG	1.T.c	6.P.t	0	-0.3240	-0.4379	-0.2101	0.00000	****
27	DGDG	1.T.c	7.SP	0	-0.3512	-0.4651	-0.2374	0.00000	****
28	DGDG	2.S.o	3.C.a	0	0.5762	0.4623	0.6900	0.00000	****
29	DGDG	2.S.o	4.C.v	0	0.1454	0.0316	0.2593	0.00598	**
30	DGDG	2.S.o	5.N.o	0	0.3819	0.2680	0.4958	0.00000	****
31	DGDG	2.S.o	6.P.t	0	0.0669	-0.0470	0.1808	0.51900	ns
32	DGDG	2.S.o	7.SP	0	0.0397	-0.0742	0.1536	0.92100	ns
33	DGDG	3.C.a	4.C.v	0	-0.4307	-0.5446	-0.3169	0.00000	****
34	DGDG	3.C.a	5.N.o	0	-0.1943	-0.3081	-0.0804	0.00017	***
35	DGDG	3.C.a	6.P.t	0	-0.5092	-0.6231	-0.3954	0.00000	****
36	DGDG	3.C.a	7.SP	0	-0.5365	-0.6503	-0.4226	0.00000	****
37	DGDG	4.C.v	5.N.o	0	0.2365	0.1226	0.3503	0.00001	****
38	DGDG	4.C.v	6.P.t	0	-0.0785	-0.1924	0.0354	0.33400	ns
39	DGDG	4.C.v	7.SP	0	-0.1057	-0.2196	0.0081	0.08200	ns
40	DGDG	5.N.o	6.P.t	0	-0.3150	-0.4289	-0.2011	0.00000	****
41	DGDG	5.N.o	7.SP	0	-0.3422	-0.4561	-0.2284	0.00000	****
42	DGDG	6.P.t	7.SP	0	-0.0272	-0.1411	0.0866	0.98700	ns
43	DGMG	1.T.c	2.S.o	0	-0.0413	-0.2278	0.1452	0.99100	ns
44	DGMG	1.T.c	3.C.a	0	1.2084	1.0219	1.3949	0.00000	****
45	DGMG	1.T.c	4.C.v	0	-0.5589	-0.7454	-0.3724	0.00000	****
46	DGMG	1.T.c	5.N.o	0	0.3441	0.1576	0.5306	0.00005	****
47	DGMG	1.T.c	6.P.t	0	0.6390	0.4525	0.8255	0.00000	****
48	DGMG	1.T.c	7.SP	0	0.7255	0.5390	0.9120	0.00000	****
49	DGMG	2.S.o	3.C.a	0	1.2497	1.0632	1.4362	0.00000	****
50	DGMG	2.S.o	4.C.v	0	-0.5176	-0.7041	-0.3311	0.00000	****
51	DGMG	2.S.o	5.N.o	0	0.3854	0.1989	0.5719	0.00001	****
52	DGMG	2.S.o	6.P.t	0	0.6803	0.4938	0.8668	0.00000	****
53	DGMG	2.S.o	7.SP	0	0.7668	0.5803	0.9534	0.00000	****
54	DGMG	3.C.a	4.C.v	0	-1.7673	-1.9538	-1.5808	0.00000	****
55	DGMG	3.C.a	5.N.o	0	-0.8643	-1.0508	-0.6778	0.00000	****

56	DGMG	3.C.a	6.P.t	0	-0.5694	-0.7559	-0.3829	0.00000	****
57	DGMG	3.C.a	7.SP	0	-0.4828	-0.6693	-0.2963	0.00000	****
58	DGMG	4.C.v	5.N.o	0	0.9030	0.7165	1.0895	0.00000	****
59	DGMG	4.C.v	6.P.t	0	1.1979	1.0114	1.3844	0.00000	****
60	DGMG	4.C.v	7.SP	0	1.2845	1.0980	1.4710	0.00000	****
61	DGMG	5.N.o	6.P.t	0	0.2949	0.1084	0.4814	0.00048	***
62	DGMG	5.N.o	7.SP	0	0.3815	0.1950	0.5680	0.00001	****
63	DGMG	6.P.t	7.SP	0	0.0865	-0.1000	0.2730	0.75800	ns
64	DGTA	1.T.c	2.S.o	0	-2.8869	-2.9932	-2.7805	0.00000	****
65	DGTA	1.T.c	3.C.a	0	-3.6767	-3.7831	-3.5704	0.00000	****
66	DGTA	1.T.c	4.C.v	0	-3.6767	-3.7831	-3.5704	0.00000	****
67	DGTA	1.T.c	5.N.o	0	-2.7773	-2.8836	-2.6709	0.00000	****
68	DGTA	1.T.c	6.P.t	0	-0.5993	-0.7057	-0.4930	0.00000	****
69	DGTA	1.T.c	7.SP	0	-3.6767	-3.7831	-3.5704	0.00000	****
70	DGTA	2.S.o	3.C.a	0	-0.7898	-0.8962	-0.6835	0.00000	****
71	DGTA	2.S.o	4.C.v	0	-0.7898	-0.8962	-0.6835	0.00000	****
72	DGTA	2.S.o	5.N.o	0	0.1096	0.0033	0.2160	0.04010	*
73	DGTA	2.S.o	6.P.t	0	2.2875	2.1812	2.3939	0.00000	****
74	DGTA	2.S.o	7.SP	0	-0.7898	-0.8962	-0.6835	0.00000	****
75	DGTA	3.C.a	4.C.v	0	0.0000	-0.1064	0.1064	1.00000	ns
76	DGTA	3.C.a	5.N.o	0	0.8995	0.7931	1.0058	0.00000	****
77	DGTA	3.C.a	6.P.t	0	3.0774	2.9710	3.1837	0.00000	****
78	DGTA	3.C.a	7.SP	0	0.0000	-0.1064	0.1064	1.00000	ns
79	DGTA	4.C.v	5.N.o	0	0.8995	0.7931	1.0058	0.00000	****
80	DGTA	4.C.v	6.P.t	0	3.0774	2.9710	3.1837	0.00000	****
81	DGTA	4.C.v	7.SP	0	0.0000	-0.1064	0.1064	1.00000	ns
82	DGTA	5.N.o	6.P.t	0	2.1779	2.0716	2.2843	0.00000	****
83	DGTA	5.N.o	7.SP	0	-0.8995	-1.0058	-0.7931	0.00000	****
84	DGTA	6.P.t	7.SP	0	-3.0774	-3.1837	-2.9710	0.00000	****
85	DGTS	1.T.c	2.S.o	0	1.8391	1.7247	1.9534	0.00000	****
86	DGTS	1.T.c	3.C.a	0	2.0131	1.8988	2.1274	0.00000	****
87	DGTS	1.T.c	4.C.v	0	0.1393	0.0250	0.2537	0.00956	**
88	DGTS	1.T.c	5.N.o	0	1.8665	1.7522	1.9809	0.00000	****
89	DGTS	1.T.c	6.P.t	0	0.3171	0.2027	0.4314	0.00000	****
90	DGTS	1.T.c	7.SP	0	0.5780	0.4636	0.6923	0.00000	****
91	DGTS	2.S.o	3.C.a	0	0.1740	0.0597	0.2884	0.00079	***
92	DGTS	2.S.o	4.C.v	0	-1.6997	-1.8141	-1.5854	0.00000	****
93	DGTS	2.S.o	5.N.o	0	0.0275	-0.0869	0.1418	0.98700	ns
94	DGTS	2.S.o	6.P.t	0	-1.5220	-1.6363	-1.4076	0.00000	****
95	DGTS	2.S.o	7.SP	0	-1.2611	-1.3755	-1.1468	0.00000	****
96	DGTS	3.C.a	4.C.v	0	-1.8738	-1.9881	-1.7594	0.00000	****
97	DGTS	3.C.a	5.N.o	0	-0.1466	-0.2609	-0.0322	0.00576	**
98	DGTS	3.C.a	6.P.t	0	-1.6960	-1.8104	-1.5817	0.00000	****
99	DGTS	3.C.a	7.SP	0	-1.4351	-1.5495	-1.3208	0.00000	****
100	DGTS	4.C.v	5.N.o	0	1.7272	1.6129	1.8416	0.00000	****
101	DGTS	4.C.v	6.P.t	0	0.1777	0.0634	0.2921	0.00060	***

102	DGTS	4.C.v	7.SP	0	0.4386	0.3243	0.5530	0.00000	****
103	DGTS	5.N.o	6.P.t	0	-1.5495	-1.6638	-1.4351	0.00000	****
104	DGTS	5.N.o	7.SP	0	-1.2886	-1.4029	-1.1743	0.00000	****
105	DGTS	6.P.t	7.SP	0	0.2609	0.1465	0.3752	0.00000	****
106	LPC	1.T.c	2.S.o	0	-0.0864	-0.2621	0.0894	0.70800	ns
107	LPC	1.T.c	3.C.a	0	0.4188	0.2431	0.5946	0.00000	****
108	LPC	1.T.c	4.C.v	0	0.5012	0.3255	0.6770	0.00000	****
109	LPC	1.T.c	5.N.o	0	0.6690	0.4933	0.8448	0.00000	****
110	LPC	1.T.c	6.P.t	0	1.0931	0.9173	1.2688	0.00000	****
111	LPC	1.T.c	7.SP	0	-1.1182	-1.2940	-0.9425	0.00000	****
112	LPC	2.S.o	3.C.a	0	0.5052	0.3295	0.6809	0.00000	****
113	LPC	2.S.o	4.C.v	0	0.5876	0.4119	0.7634	0.00000	****
114	LPC	2.S.o	5.N.o	0	0.7554	0.5797	0.9312	0.00000	****
115	LPC	2.S.o	6.P.t	0	1.1795	1.0037	1.3552	0.00000	****
116	LPC	2.S.o	7.SP	0	-1.0319	-1.2076	-0.8561	0.00000	****
117	LPC	3.C.a	4.C.v	0	0.0824	-0.0933	0.2582	0.74900	ns
118	LPC	3.C.a	5.N.o	0	0.2502	0.0745	0.4260	0.00179	**
119	LPC	3.C.a	6.P.t	0	0.6743	0.4985	0.8500	0.00000	****
120	LPC	3.C.a	7.SP	0	-1.5371	-1.7128	-1.3613	0.00000	****
121	LPC	4.C.v	5.N.o	0	0.1678	-0.0079	0.3435	0.06860	ns
122	LPC	4.C.v	6.P.t	0	0.5918	0.4161	0.7676	0.00000	****
123	LPC	4.C.v	7.SP	0	-1.6195	-1.7952	-1.4437	0.00000	****
124	LPC	5.N.o	6.P.t	0	0.4240	0.2483	0.5998	0.00000	****
125	LPC	5.N.o	7.SP	0	-1.7873	-1.9630	-1.6115	0.00000	****
126	LPC	6.P.t	7.SP	0	-2.2113	-2.3871	-2.0356	0.00000	****
127	LPE	1.T.c	2.S.o	0	1.2839	1.0345	1.5334	0.00000	****
128	LPE	1.T.c	3.C.a	0	1.0488	0.7993	1.2982	0.00000	****
129	LPE	1.T.c	4.C.v	0	1.9055	1.6560	2.1550	0.00000	****
130	LPE	1.T.c	5.N.o	0	1.1982	0.9488	1.4477	0.00000	****
131	LPE	1.T.c	6.P.t	0	1.2696	1.0201	1.5191	0.00000	****
132	LPE	1.T.c	7.SP	0	-0.9581	-1.2075	-0.7086	0.00000	****
133	LPE	2.S.o	3.C.a	0	-0.2352	-0.4847	0.0143	0.07450	ns
134	LPE	2.S.o	4.C.v	0	0.6215	0.3721	0.8710	0.00000	****
135	LPE	2.S.o	5.N.o	0	-0.0857	-0.3352	0.1638	0.92600	ns
136	LPE	2.S.o	6.P.t	0	-0.0143	-0.2638	0.2351	1.00000	ns
137	LPE	2.S.o	7.SP	0	-2.2420	-2.4915	-1.9925	0.00000	****
138	LPE	3.C.a	4.C.v	0	0.8567	0.6072	1.1062	0.00000	****
139	LPE	3.C.a	5.N.o	0	0.1495	-0.1000	0.3990	0.49600	ns
140	LPE	3.C.a	6.P.t	0	0.2208	-0.0286	0.4703	0.10900	ns
141	LPE	3.C.a	7.SP	0	-2.0068	-2.2563	-1.7573	0.00000	****
142	LPE	4.C.v	5.N.o	0	-0.7072	-0.9567	-0.4578	0.00000	****
143	LPE	4.C.v	6.P.t	0	-0.6359	-0.8854	-0.3864	0.00000	****
144	LPE	4.C.v	7.SP	0	-2.8635	-3.1130	-2.6141	0.00000	****
145	LPE	5.N.o	6.P.t	0	0.0714	-0.1781	0.3208	0.96800	ns
146	LPE	5.N.o	7.SP	0	-2.1563	-2.4058	-1.9068	0.00000	****
147	LPE	6.P.t	7.SP	0	-2.2277	-2.4771	-1.9782	0.00000	****

148	LPG	1.T.c	2.S.o	0	1.2845	0.8798	1.6892	0.00000	****
149	LPG	1.T.c	3.C.a	0	1.5537	1.1490	1.9584	0.00000	****
150	LPG	1.T.c	4.C.v	0	1.6551	1.2504	2.0598	0.00000	****
151	LPG	1.T.c	5.N.o	0	1.7903	1.3855	2.1950	0.00000	****
152	LPG	1.T.c	6.P.t	0	1.6254	1.2207	2.0301	0.00000	****
153	LPG	1.T.c	7.SP	0	2.7621	2.3574	3.1668	0.00000	****
154	LPG	2.S.o	3.C.a	0	0.2692	-0.1356	0.6739	0.37500	ns
155	LPG	2.S.o	4.C.v	0	0.3706	-0.0342	0.7753	0.08950	ns
156	LPG	2.S.o	5.N.o	0	0.5057	0.1010	0.9105	0.00746	**
157	LPG	2.S.o	6.P.t	0	0.3409	-0.0638	0.7456	0.14300	ns
158	LPG	2.S.o	7.SP	0	1.4776	1.0728	1.8823	0.00000	****
159	LPG	3.C.a	4.C.v	0	0.1014	-0.3033	0.5061	0.98400	ns
160	LPG	3.C.a	5.N.o	0	0.2366	-0.1682	0.6413	0.52500	ns
161	LPG	3.C.a	6.P.t	0	0.0717	-0.3330	0.4765	0.99700	ns
162	LPG	3.C.a	7.SP	0	1.2084	0.8037	1.6131	0.00000	****
163	LPG	4.C.v	5.N.o	0	0.1352	-0.2696	0.5399	0.93500	ns
164	LPG	4.C.v	6.P.t	0	-0.0297	-0.4344	0.3751	1.00000	ns
165	LPG	4.C.v	7.SP	0	1.1070	0.7023	1.5117	0.00000	****
166	LPG	5.N.o	6.P.t	0	-0.1648	-0.5696	0.2399	0.85000	ns
167	LPG	5.N.o	7.SP	0	0.9718	0.5671	1.3765	0.00000	****
168	LPG	6.P.t	7.SP	0	1.1367	0.7319	1.5414	0.00000	****
169	MGDG	1.T.c	2.S.o	0	-0.2323	-0.3527	-0.1118	0.00003	****
170	MGDG	1.T.c	3.C.a	0	-0.2452	-0.3657	-0.1248	0.00001	****
171	MGDG	1.T.c	4.C.v	0	-0.1583	-0.2788	-0.0379	0.00441	**
172	MGDG	1.T.c	5.N.o	0	-0.3914	-0.5118	-0.2710	0.00000	****
173	MGDG	1.T.c	6.P.t	0	-0.1535	-0.2740	-0.0331	0.00610	**
174	MGDG	1.T.c	7.SP	0	-0.2543	-0.3748	-0.1339	0.00001	****
175	MGDG	2.S.o	3.C.a	0	-0.0130	-0.1334	0.1075	1.00000	ns
176	MGDG	2.S.o	4.C.v	0	0.0740	-0.0465	0.1944	0.46800	ns
177	MGDG	2.S.o	5.N.o	0	-0.1591	-0.2796	-0.0387	0.00418	**
178	MGDG	2.S.o	6.P.t	0	0.0787	-0.0417	0.1992	0.39500	ns
179	MGDG	2.S.o	7.SP	0	-0.0220	-0.1425	0.0984	0.99700	ns
180	MGDG	3.C.a	4.C.v	0	0.0869	-0.0335	0.2074	0.28400	ns
181	MGDG	3.C.a	5.N.o	0	-0.1462	-0.2666	-0.0257	0.00994	**
182	MGDG	3.C.a	6.P.t	0	0.0917	-0.0288	0.2121	0.23000	ns
183	MGDG	3.C.a	7.SP	0	-0.0091	-0.1295	0.1114	1.00000	ns
184	MGDG	4.C.v	5.N.o	0	-0.2331	-0.3535	-0.1126	0.00002	****
185	MGDG	4.C.v	6.P.t	0	0.0048	-0.1157	0.1252	1.00000	ns
186	MGDG	4.C.v	7.SP	0	-0.0960	-0.2164	0.0245	0.18800	ns
187	MGDG	5.N.o	6.P.t	0	0.2379	0.1174	0.3583	0.00002	****
188	MGDG	5.N.o	7.SP	0	0.1371	0.0166	0.2575	0.01790	*
189	MGDG	6.P.t	7.SP	0	-0.1008	-0.2212	0.0197	0.14800	ns
190	MGMG	1.T.c	2.S.o	0	0.4137	0.2401	0.5873	0.00000	****
191	MGMG	1.T.c	3.C.a	0	1.0915	0.9178	1.2651	0.00000	****
192	MGMG	1.T.c	4.C.v	0	-0.4675	-0.6411	-0.2939	0.00000	****
193	MGMG	1.T.c	5.N.o	0	-0.1875	-0.3611	-0.0138	0.02790	*

194	MGMG	1.T.c	6.P.t	0	0.1865	0.0129	0.3601	0.02910	*
195	MGMG	1.T.c	7.SP	0	0.5014	0.3278	0.6750	0.00000	****
196	MGMG	2.S.o	3.C.a	0	0.6778	0.5041	0.8514	0.00000	****
197	MGMG	2.S.o	4.C.v	0	-0.8812	-1.0548	-0.7076	0.00000	****
198	MGMG	2.S.o	5.N.o	0	-0.6012	-0.7748	-0.4275	0.00000	****
199	MGMG	2.S.o	6.P.t	0	-0.2272	-0.4008	-0.0536	0.00464	**
200	MGMG	2.S.o	7.SP	0	0.0877	-0.0859	0.2614	0.68200	ns
201	MGMG	3.C.a	4.C.v	0	-1.5590	-1.7326	-1.3853	0.00000	****
202	MGMG	3.C.a	5.N.o	0	-1.2789	-1.4526	-1.1053	0.00000	****
203	MGMG	3.C.a	6.P.t	0	-0.9050	-1.0786	-0.7313	0.00000	****
204	MGMG	3.C.a	7.SP	0	-0.5900	-0.7637	-0.4164	0.00000	****
205	MGMG	4.C.v	5.N.o	0	0.2800	0.1064	0.4537	0.00037	***
206	MGMG	4.C.v	6.P.t	0	0.6540	0.4804	0.8276	0.00000	****
207	MGMG	4.C.v	7.SP	0	0.9689	0.7953	1.1426	0.00000	****
208	MGMG	5.N.o	6.P.t	0	0.3740	0.2003	0.5476	0.00000	****
209	MGMG	5.N.o	7.SP	0	0.6889	0.5153	0.8625	0.00000	****
210	MGMG	6.P.t	7.SP	0	0.3149	0.1413	0.4885	0.00007	****
211	MGTA	1.T.c	2.S.o	0	-0.9140	-1.0098	-0.8182	0.00000	****
212	MGTA	1.T.c	3.C.a	0	-0.9140	-1.0098	-0.8182	0.00000	****
213	MGTA	1.T.c	4.C.v	0	-0.9140	-1.0098	-0.8182	0.00000	****
214	MGTA	1.T.c	5.N.o	0	-0.9140	-1.0098	-0.8182	0.00000	****
215	MGTA	1.T.c	6.P.t	0	-0.0753	-0.1710	0.0205	0.20000	ns
216	MGTA	1.T.c	7.SP	0	-0.9140	-1.0098	-0.8182	0.00000	****
217	MGTA	2.S.o	3.C.a	0	0.0000	-0.0958	0.0958	1.00000	ns
218	MGTA	2.S.o	4.C.v	0	0.0000	-0.0958	0.0958	1.00000	ns
219	MGTA	2.S.o	5.N.o	0	0.0000	-0.0958	0.0958	1.00000	ns
220	MGTA	2.S.o	6.P.t	0	0.8388	0.7430	0.9345	0.00000	****
221	MGTA	2.S.o	7.SP	0	0.0000	-0.0958	0.0958	1.00000	ns
222	MGTA	3.C.a	4.C.v	0	0.0000	-0.0958	0.0958	1.00000	ns
223	MGTA	3.C.a	5.N.o	0	0.0000	-0.0958	0.0958	1.00000	ns
224	MGTA	3.C.a	6.P.t	0	0.8388	0.7430	0.9345	0.00000	****
225	MGTA	3.C.a	7.SP	0	0.0000	-0.0958	0.0958	1.00000	ns
226	MGTA	4.C.v	5.N.o	0	0.0000	-0.0958	0.0958	1.00000	ns
227	MGTA	4.C.v	6.P.t	0	0.8388	0.7430	0.9345	0.00000	****
228	MGTA	4.C.v	7.SP	0	0.0000	-0.0958	0.0958	1.00000	ns
229	MGTA	5.N.o	6.P.t	0	0.8388	0.7430	0.9345	0.00000	****
230	MGTA	5.N.o	7.SP	0	0.0000	-0.0958	0.0958	1.00000	ns
231	MGTA	6.P.t	7.SP	0	-0.8388	-0.9345	-0.7430	0.00000	****
232	MGTS	1.T.c	2.S.o	0	1.4878	1.3370	1.6386	0.00000	****
233	MGTS	1.T.c	3.C.a	0	2.2917	2.1409	2.4425	0.00000	****
234	MGTS	1.T.c	4.C.v	0	0.7217	0.5709	0.8725	0.00000	****
235	MGTS	1.T.c	5.N.o	0	1.5016	1.3508	1.6524	0.00000	****
236	MGTS	1.T.c	6.P.t	0	0.0222	-0.1286	0.1730	0.99900	ns
237	MGTS	1.T.c	7.SP	0	0.5513	0.4005	0.7021	0.00000	****
238	MGTS	2.S.o	3.C.a	0	0.8039	0.6531	0.9547	0.00000	****
239	MGTS	2.S.o	4.C.v	0	-0.7661	-0.9169	-0.6153	0.00000	****

240	MGTS	2.S.o	5.N.o	0	0.0138	-0.1370	0.1646	1.00000	ns
241	MGTS	2.S.o	6.P.t	0	-1.4656	-1.6164	-1.3148	0.00000	****
242	MGTS	2.S.o	7.SP	0	-0.9364	-1.0872	-0.7856	0.00000	****
243	MGTS	3.C.a	4.C.v	0	-1.5700	-1.7208	-1.4192	0.00000	****
244	MGTS	3.C.a	5.N.o	0	-0.7901	-0.9409	-0.6393	0.00000	****
245	MGTS	3.C.a	6.P.t	0	-2.2695	-2.4203	-2.1187	0.00000	****
246	MGTS	3.C.a	7.SP	0	-1.7404	-1.8912	-1.5896	0.00000	****
247	MGTS	4.C.v	5.N.o	0	0.7799	0.6291	0.9307	0.00000	****
248	MGTS	4.C.v	6.P.t	0	-0.6995	-0.8503	-0.5487	0.00000	****
249	MGTS	4.C.v	7.SP	0	-0.1704	-0.3212	-0.0196	0.01910	*
250	MGTS	5.N.o	6.P.t	0	-1.4794	-1.6302	-1.3286	0.00000	****
251	MGTS	5.N.o	7.SP	0	-0.9502	-1.1010	-0.7994	0.00000	****
252	MGTS	6.P.t	7.SP	0	0.5291	0.3784	0.6799	0.00000	****
253	PC	1.T.c	2.S.o	0	-0.1145	-0.2364	0.0074	0.07630	ns
254	PC	1.T.c	3.C.a	0	0.0601	-0.0618	0.1820	0.70500	ns
255	PC	1.T.c	4.C.v	0	0.4685	0.3466	0.5904	0.00000	****
256	PC	1.T.c	5.N.o	0	0.3923	0.2704	0.5141	0.00000	****
257	PC	1.T.c	6.P.t	0	0.2456	0.1237	0.3675	0.00001	****
258	PC	1.T.c	7.SP	0	-1.4330	-1.5549	-1.3112	0.00000	****
259	PC	2.S.o	3.C.a	0	0.1746	0.0527	0.2965	0.00166	**
260	PC	2.S.o	4.C.v	0	0.5830	0.4611	0.7049	0.00000	****
261	PC	2.S.o	5.N.o	0	0.5067	0.3849	0.6286	0.00000	****
262	PC	2.S.o	6.P.t	0	0.3601	0.2382	0.4819	0.00000	****
263	PC	2.S.o	7.SP	0	-1.3186	-1.4404	-1.1967	0.00000	****
264	PC	3.C.a	4.C.v	0	0.4084	0.2865	0.5303	0.00000	****
265	PC	3.C.a	5.N.o	0	0.3321	0.2103	0.4540	0.00000	****
266	PC	3.C.a	6.P.t	0	0.1855	0.0636	0.3073	0.00079	***
267	PC	3.C.a	7.SP	0	-1.4932	-1.6150	-1.3713	0.00000	****
268	PC	4.C.v	5.N.o	0	-0.0762	-0.1981	0.0456	0.44600	ns
269	PC	4.C.v	6.P.t	0	-0.2229	-0.3448	-0.1010	0.00006	****
270	PC	4.C.v	7.SP	0	-1.9015	-2.0234	-1.7797	0.00000	****
271	PC	5.N.o	6.P.t	0	-0.1467	-0.2686	-0.0248	0.01080	*
272	PC	5.N.o	7.SP	0	-1.8253	-1.9472	-1.7034	0.00000	****
273	PC	6.P.t	7.SP	0	-1.6786	-1.8005	-1.5568	0.00000	****
274	PE	1.T.c	2.S.o	0	1.0351	0.7992	1.2711	0.00000	****
275	PE	1.T.c	3.C.a	0	0.2104	-0.0256	0.4464	0.10500	ns
276	PE	1.T.c	4.C.v	0	1.3130	1.0770	1.5490	0.00000	****
277	PE	1.T.c	5.N.o	0	0.5022	0.2662	0.7382	0.00000	****
278	PE	1.T.c	6.P.t	0	0.6665	0.4305	0.9024	0.00000	****
279	PE	1.T.c	7.SP	0	-0.4360	-0.6720	-0.2000	0.00005	****
280	PE	2.S.o	3.C.a	0	-0.8247	-1.0607	-0.5887	0.00000	****
281	PE	2.S.o	4.C.v	0	0.2779	0.0419	0.5139	0.01320	*
282	PE	2.S.o	5.N.o	0	-0.5329	-0.7689	-0.2969	0.00000	****
283	PE	2.S.o	6.P.t	0	-0.3687	-0.6047	-0.1327	0.00056	***
284	PE	2.S.o	7.SP	0	-1.4712	-1.7072	-1.2352	0.00000	****
285	PE	3.C.a	4.C.v	0	1.1026	0.8666	1.3386	0.00000	****

286	PE	3.C.a	5.N.o	0	0.2918	0.0558	0.5278	0.00828	**
287	PE	3.C.a	6.P.t	0	0.4560	0.2200	0.6920	0.00002	****
288	PE	3.C.a	7.SP	0	-0.6465	-0.8825	-0.4105	0.00000	****
289	PE	4.C.v	5.N.o	0	-0.8108	-1.0468	-0.5748	0.00000	****
290	PE	4.C.v	6.P.t	0	-0.6466	-0.8826	-0.4106	0.00000	****
291	PE	4.C.v	7.SP	0	-1.7491	-1.9851	-1.5131	0.00000	****
292	PE	5.N.o	6.P.t	0	0.1642	-0.0718	0.4002	0.32300	ns
293	PE	5.N.o	7.SP	0	-0.9383	-1.1743	-0.7023	0.00000	****
294	PE	6.P.t	7.SP	0	-1.1025	-1.3385	-0.8665	0.00000	****
295	PG	1.T.c	2.S.o	0	0.0543	-0.0936	0.2022	0.90100	ns
296	PG	1.T.c	3.C.a	0	-0.0308	-0.1787	0.1171	0.99400	ns
297	PG	1.T.c	4.C.v	0	0.3147	0.1668	0.4625	0.00000	****
298	PG	1.T.c	5.N.o	0	-0.1021	-0.2500	0.0458	0.33200	ns
299	PG	1.T.c	6.P.t	0	-0.0542	-0.2021	0.0937	0.90200	ns
300	PG	1.T.c	7.SP	0	0.4177	0.2698	0.5656	0.00000	****
301	PG	2.S.o	3.C.a	0	-0.0851	-0.2330	0.0628	0.54300	ns
302	PG	2.S.o	4.C.v	0	0.2604	0.1125	0.4083	0.00010	***
303	PG	2.S.o	5.N.o	0	-0.1564	-0.3043	-0.0085	0.03300	*
304	PG	2.S.o	6.P.t	0	-0.1084	-0.2563	0.0394	0.26700	ns
305	PG	2.S.o	7.SP	0	0.3634	0.2155	0.5113	0.00000	****
306	PG	3.C.a	4.C.v	0	0.3455	0.1976	0.4934	0.00000	****
307	PG	3.C.a	5.N.o	0	-0.0713	-0.2191	0.0766	0.72600	ns
308	PG	3.C.a	6.P.t	0	-0.0233	-0.1712	0.1245	0.99900	ns
309	PG	3.C.a	7.SP	0	0.4485	0.3006	0.5964	0.00000	****
310	PG	4.C.v	5.N.o	0	-0.4167	-0.5646	-0.2689	0.00000	****
311	PG	4.C.v	6.P.t	0	-0.3688	-0.5167	-0.2209	0.00000	****
312	PG	4.C.v	7.SP	0	0.1030	-0.0449	0.2509	0.32200	ns
313	PG	5.N.o	6.P.t	0	0.0479	-0.1000	0.1958	0.94300	ns
314	PG	5.N.o	7.SP	0	0.5198	0.3719	0.6676	0.00000	****
315	PG	6.P.t	7.SP	0	0.4718	0.3240	0.6197	0.00000	****
316	PI	1.T.c	2.S.o	0	-0.5183	-0.7280	-0.3085	0.00000	****
317	PI	1.T.c	3.C.a	0	0.3697	0.1599	0.5794	0.00010	***
318	PI	1.T.c	4.C.v	0	-0.1487	-0.3585	0.0611	0.30300	ns
319	PI	1.T.c	5.N.o	0	0.3592	0.1494	0.5690	0.00016	***
320	PI	1.T.c	6.P.t	0	0.2848	0.0750	0.4945	0.00310	**
321	PI	1.T.c	7.SP	0	-1.4119	-1.6217	-1.2021	0.00000	****
322	PI	2.S.o	3.C.a	0	0.8879	0.6781	1.0977	0.00000	****
323	PI	2.S.o	4.C.v	0	0.3695	0.1598	0.5793	0.00010	***
324	PI	2.S.o	5.N.o	0	0.8774	0.6676	1.0872	0.00000	****
325	PI	2.S.o	6.P.t	0	0.8030	0.5932	1.0128	0.00000	****
326	PI	2.S.o	7.SP	0	-0.8937	-1.1035	-0.6839	0.00000	****
327	PI	3.C.a	4.C.v	0	-0.5184	-0.7282	-0.3086	0.00000	****
328	PI	3.C.a	5.N.o	0	-0.0105	-0.2203	0.1993	1.00000	ns
329	PI	3.C.a	6.P.t	0	-0.0849	-0.2947	0.1249	0.85400	ns
330	PI	3.C.a	7.SP	0	-1.7816	-1.9914	-1.5718	0.00000	****
331	PI	4.C.v	5.N.o	0	0.5079	0.2981	0.7177	0.00000	****

332	PI	4.C.v	6.P.t	0	0.4335	0.2237	0.6433	0.00001	****
333	PI	4.C.v	7.SP	0	-1.2632	-1.4730	-1.0534	0.00000	****
334	PI	5.N.o	6.P.t	0	-0.0744	-0.2842	0.1354	0.91500	ns
335	PI	5.N.o	7.SP	0	-1.7711	-1.9809	-1.5613	0.00000	****
336	PI	6.P.t	7.SP	0	-1.6967	-1.9065	-1.4869	0.00000	****
337	PI-Cer	1.T.c	2.S.o	0	0.0000	-0.1705	0.1705	1.00000	ns
338	PI-Cer	1.T.c	3.C.a	0	2.6373	2.4668	2.8079	0.00000	****
339	PI-Cer	1.T.c	4.C.v	0	2.1788	2.0083	2.3494	0.00000	****
340	PI-Cer	1.T.c	5.N.o	0	3.0246	2.8541	3.1952	0.00000	****
341	PI-Cer	1.T.c	6.P.t	0	0.9024	0.7319	1.0729	0.00000	****
342	PI-Cer	1.T.c	7.SP	0	2.2207	2.0502	2.3912	0.00000	****
343	PI-Cer	2.S.o	3.C.a	0	2.6373	2.4668	2.8079	0.00000	****
344	PI-Cer	2.S.o	4.C.v	0	2.1788	2.0083	2.3494	0.00000	****
345	PI-Cer	2.S.o	5.N.o	0	3.0246	2.8541	3.1952	0.00000	****
346	PI-Cer	2.S.o	6.P.t	0	0.9024	0.7319	1.0729	0.00000	****
347	PI-Cer	2.S.o	7.SP	0	2.2207	2.0502	2.3912	0.00000	****
348	PI-Cer	3.C.a	4.C.v	0	-0.4585	-0.6290	-0.2880	0.00000	****
349	PI-Cer	3.C.a	5.N.o	0	0.3873	0.2168	0.5579	0.00000	****
350	PI-Cer	3.C.a	6.P.t	0	-1.7349	-1.9054	-1.5644	0.00000	****
351	PI-Cer	3.C.a	7.SP	0	-0.4166	-0.5872	-0.2461	0.00000	****
352	PI-Cer	4.C.v	5.N.o	0	0.8458	0.6753	1.0164	0.00000	****
353	PI-Cer	4.C.v	6.P.t	0	-1.2764	-1.4469	-1.1059	0.00000	****
354	PI-Cer	4.C.v	7.SP	0	0.0419	-0.1287	0.2124	0.98500	ns
355	PI-Cer	5.N.o	6.P.t	0	-2.1222	-2.2928	-1.9517	0.00000	****
356	PI-Cer	5.N.o	7.SP	0	-0.8040	-0.9745	-0.6334	0.00000	****
357	PI-Cer	6.P.t	7.SP	0	1.3183	1.1477	1.4888	0.00000	****
358	SQDG	1.T.c	2.S.o	0	-0.2304	-0.3850	-0.0758	0.00102	**
359	SQDG	1.T.c	3.C.a	0	0.0673	-0.0873	0.2219	0.80700	ns
360	SQDG	1.T.c	4.C.v	0	-0.5186	-0.6731	-0.3640	0.00000	****
361	SQDG	1.T.c	5.N.o	0	-0.1052	-0.2598	0.0494	0.34800	ns
362	SQDG	1.T.c	6.P.t	0	0.1136	-0.0409	0.2682	0.26500	ns
363	SQDG	1.T.c	7.SP	0	0.2238	0.0692	0.3783	0.00147	**
364	SQDG	2.S.o	3.C.a	0	0.2977	0.1432	0.4523	0.00003	****
365	SQDG	2.S.o	4.C.v	0	-0.2882	-0.4427	-0.1336	0.00004	****
366	SQDG	2.S.o	5.N.o	0	0.1252	-0.0294	0.2798	0.17400	ns
367	SQDG	2.S.o	6.P.t	0	0.3440	0.1895	0.4986	0.00000	****
368	SQDG	2.S.o	7.SP	0	0.4542	0.2996	0.6087	0.00000	****
369	SQDG	3.C.a	4.C.v	0	-0.5859	-0.7405	-0.4313	0.00000	****
370	SQDG	3.C.a	5.N.o	0	-0.1725	-0.3271	-0.0180	0.02120	*
371	SQDG	3.C.a	6.P.t	0	0.0463	-0.1083	0.2009	0.96000	ns
372	SQDG	3.C.a	7.SP	0	0.1564	0.0019	0.3110	0.04590	*
373	SQDG	4.C.v	5.N.o	0	0.4133	0.2588	0.5679	0.00000	****
374	SQDG	4.C.v	6.P.t	0	0.6322	0.4776	0.7868	0.00000	****
375	SQDG	4.C.v	7.SP	0	0.7423	0.5877	0.8969	0.00000	****
376	SQDG	5.N.o	6.P.t	0	0.2189	0.0643	0.3734	0.00191	**
377	SQDG	5.N.o	7.SP	0	0.3290	0.1744	0.4835	0.00000	****

378	SQDG	6.P.t	7.SP	0	0.1101	-0.0445	0.2647	0.29800	ns
379	SQMG	1.T.c	2.S.o	0	0.0000	-0.2409	0.2409	1.00000	ns
380	SQMG	1.T.c	3.C.a	0	0.8651	0.6242	1.1060	0.00000	****
381	SQMG	1.T.c	4.C.v	0	0.0000	-0.2409	0.2409	1.00000	ns
382	SQMG	1.T.c	5.N.o	0	0.0000	-0.2409	0.2409	1.00000	ns
383	SQMG	1.T.c	6.P.t	0	1.4666	1.2257	1.7076	0.00000	****
384	SQMG	1.T.c	7.SP	0	1.6627	1.4218	1.9037	0.00000	****
385	SQMG	2.S.o	3.C.a	0	0.8651	0.6242	1.1060	0.00000	****
386	SQMG	2.S.o	4.C.v	0	0.0000	-0.2409	0.2409	1.00000	ns
387	SQMG	2.S.o	5.N.o	0	0.0000	-0.2409	0.2409	1.00000	ns
388	SQMG	2.S.o	6.P.t	0	1.4666	1.2257	1.7076	0.00000	****
389	SQMG	2.S.o	7.SP	0	1.6627	1.4218	1.9037	0.00000	****
390	SQMG	3.C.a	4.C.v	0	-0.8651	-1.1060	-0.6242	0.00000	****
391	SQMG	3.C.a	5.N.o	0	-0.8651	-1.1060	-0.6242	0.00000	****
392	SQMG	3.C.a	6.P.t	0	0.6016	0.3606	0.8425	0.00000	****
393	SQMG	3.C.a	7.SP	0	0.7977	0.5567	1.0386	0.00000	****
394	SQMG	4.C.v	5.N.o	0	0.0000	-0.2409	0.2409	1.00000	ns
395	SQMG	4.C.v	6.P.t	0	1.4666	1.2257	1.7076	0.00000	****
396	SQMG	4.C.v	7.SP	0	1.6627	1.4218	1.9037	0.00000	****
397	SQMG	5.N.o	6.P.t	0	1.4666	1.2257	1.7076	0.00000	****
398	SQMG	5.N.o	7.SP	0	1.6627	1.4218	1.9037	0.00000	****
399	SQMG	6.P.t	7.SP	0	0.1961	-0.0448	0.4370	0.17000	ns

Abbreviations: DGDG, digalactosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; SQMG, sulfoquinovosylmonoacylglycerol; DGTS, diacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; MGTS, monoacylglyceryl 3-O-4'-(N,N,N-trimethyl) homoserine; DGTA, diacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; MGTA, monoacylglycerylhydroxymethyl-N,N,N-trimethyl-β-alanine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PI, phosphatidylinositol; PI-Cer, inositolphosphoceramide; Cer, ceramide

Supplementary material of Chapter 7

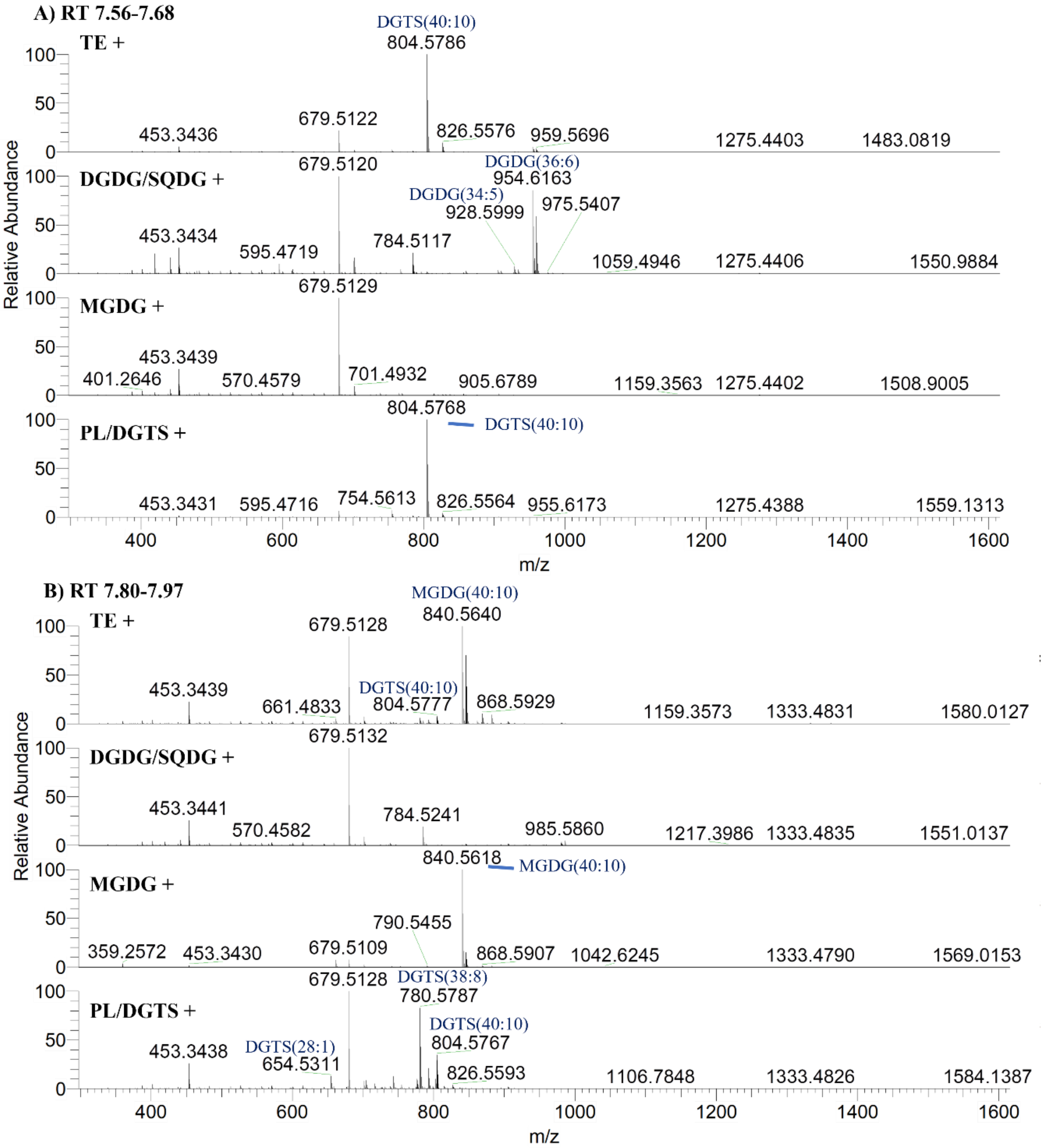
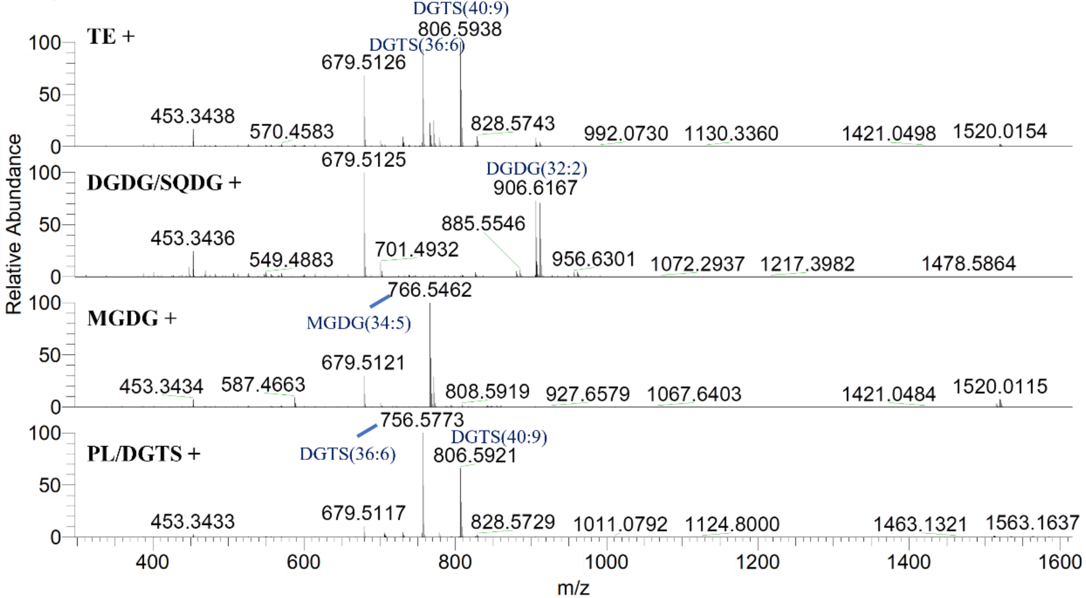


Figure S7.1. HILIC-ESI–MS spectra of the polar lipid species of *N. oceanica* identified in positive ion mode from elution time 7.56-7.68 (**A**) and 7.80-7.97 (**B**) in the total lipid extract and fractions (DGDG/SQDG; MGDG; PL/DGTS), after solid phase extraction.

A) RT 8.34-8.53



B) RT 8.64-8.74

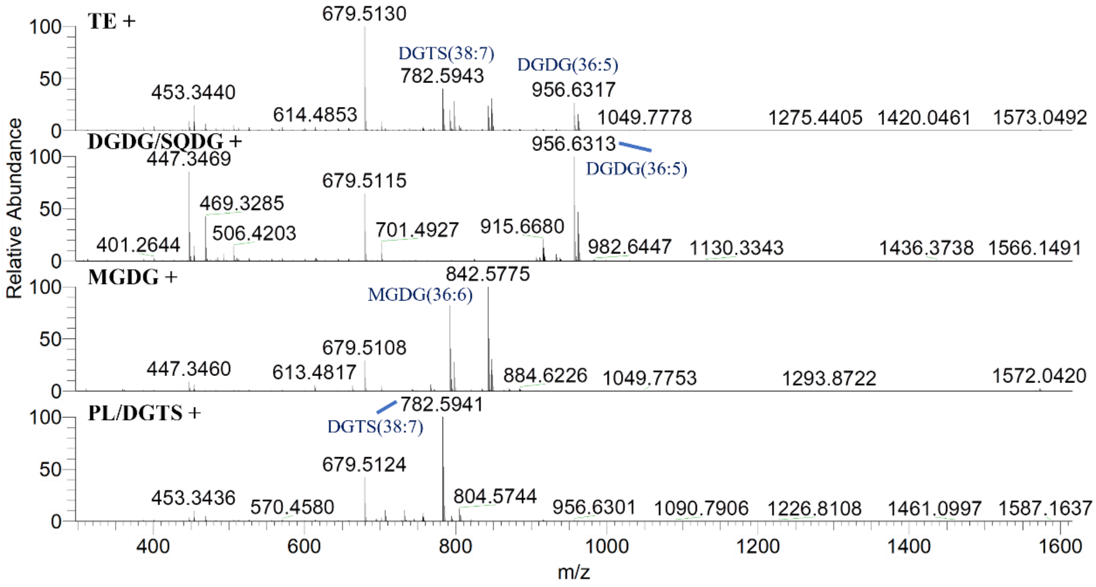
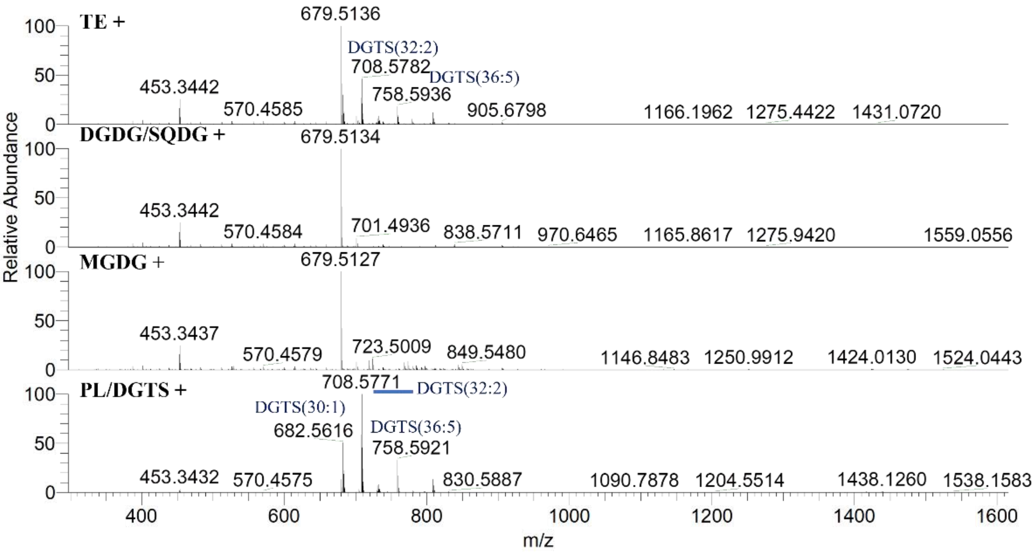


Figure S7.2. HILIC-ESI–MS spectra of the polar lipid species of *N. oceanica* identified in positive ion mode from elution time 8.34-8.53 (**A**) and 8.64-8.74 (**B**) in the total lipid extract and fractions (DGDG/SQDG; MGDG; PL/DGTS), after solid phase extraction.

A) RT 9.13-9.37



B) RT 9.47-9.68

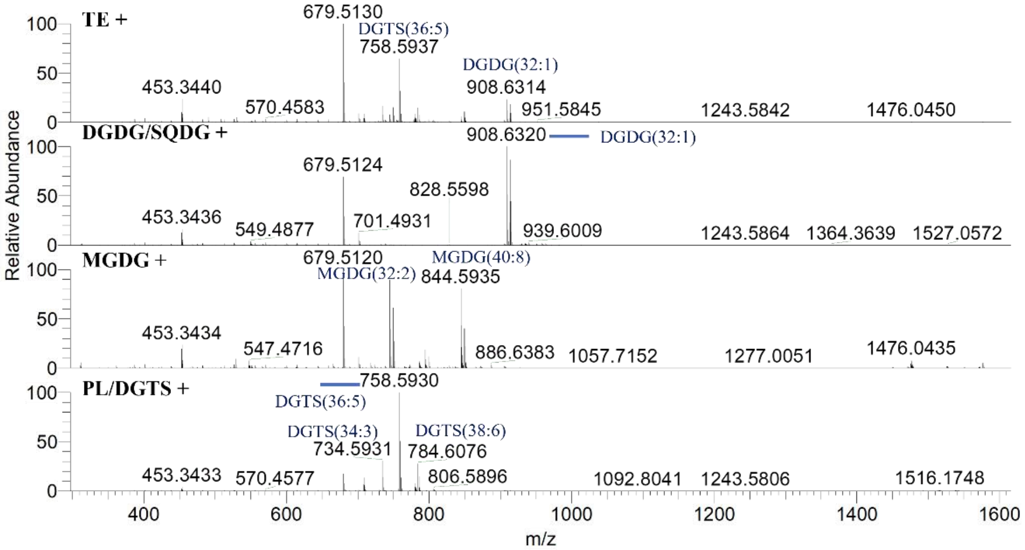


Figure S7.3. HILIC-ESI–MS spectra of the polar lipid species of *N. oceanica* identified in positive ion mode from elution time 9.13-9.37 (**A**) and 9.47-9.68 (**B**) in the total lipid extract and fractions (DGDG/SQDG; MGDG; PL/DGTS), after solid phase extraction.

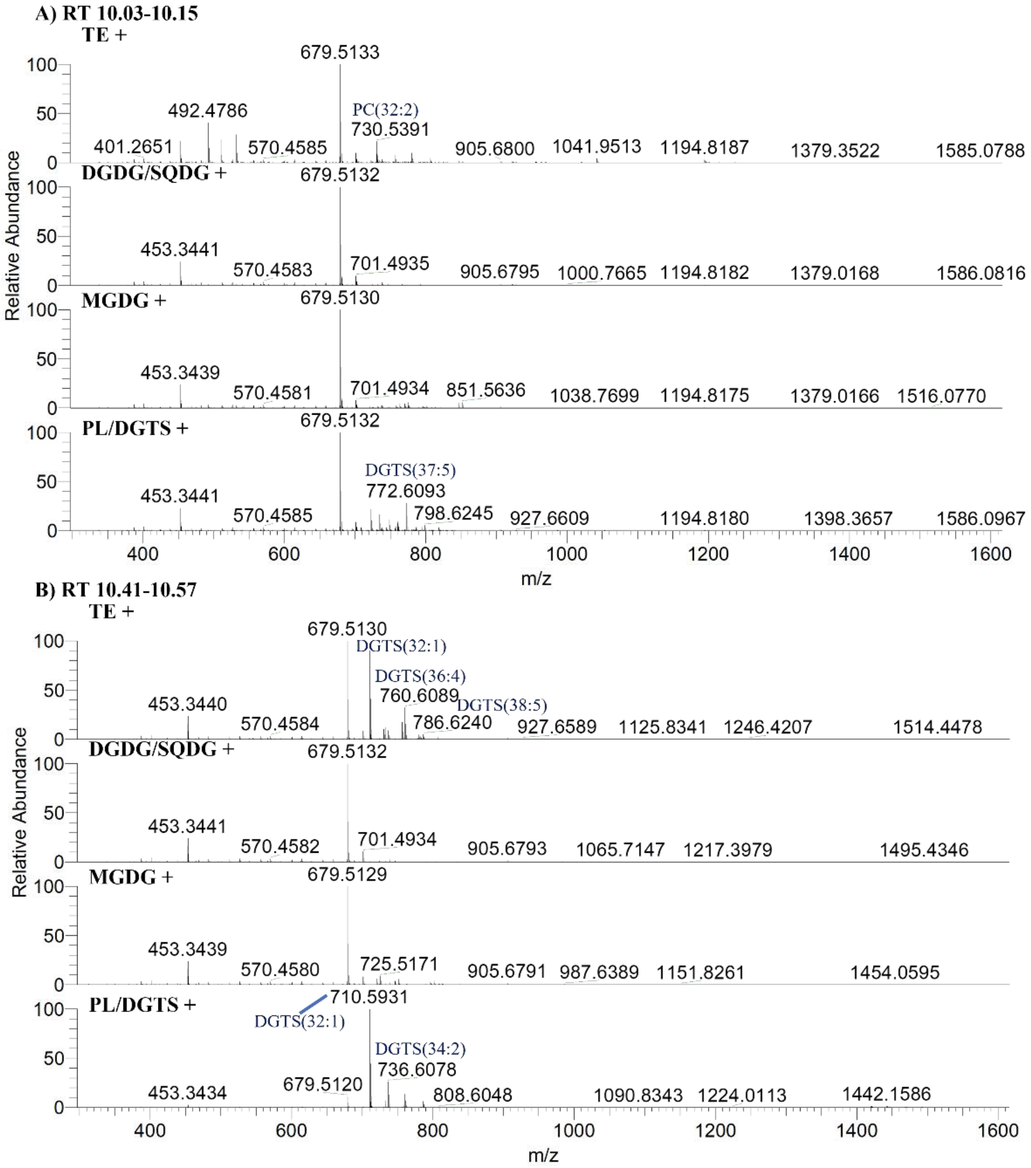
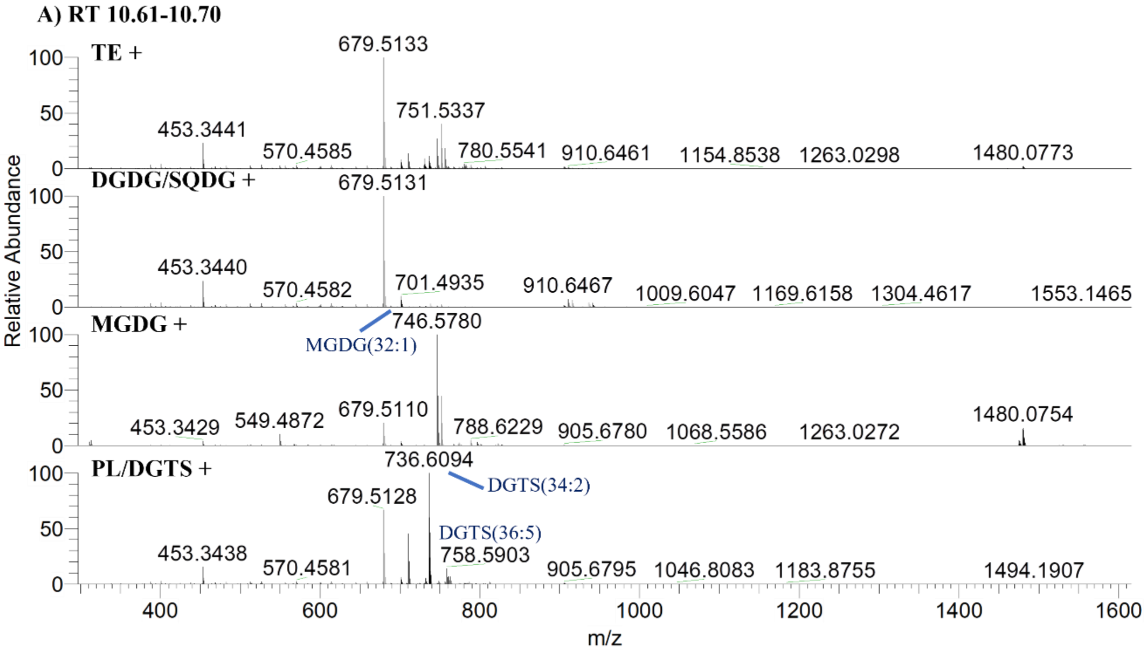
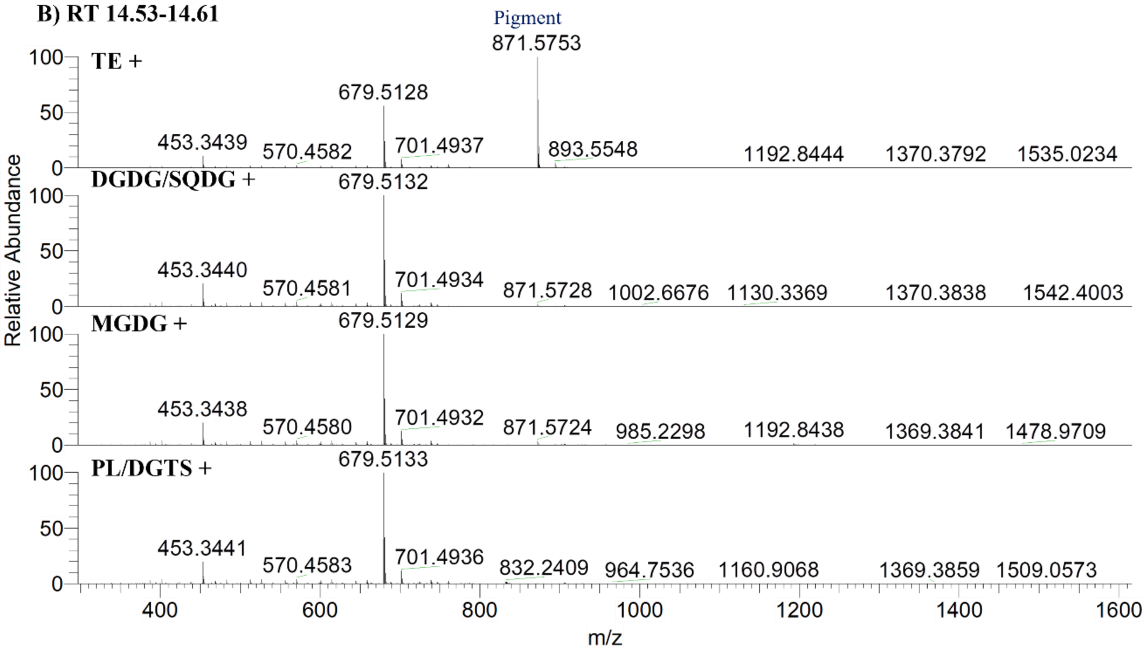


Figure S7.4. HILIC-ESI–MS spectra of the polar lipid species of *N. oceanica* identified in positive ion mode from elution time 10.03-10.15 (**A**) and 10.41-10.57 (**B**) in the total lipid extract and fractions (DGDG/SQDG; MGDG; PL/DGTS), after solid phase extraction.



A) RT 10.61-10.70



B) RT 14.53-14.61

Figure S7.5. HILIC-ESI–MS spectra of the polar lipid species of *N. oceanica* identified in positive ion mode from elution time 10.61-10.70 (**A**) and 14.53-14.61 (**B**) in the total lipid extract and fractions (DGDG/SQDG; MGDG; PL/DGTS), after solid phase extraction.

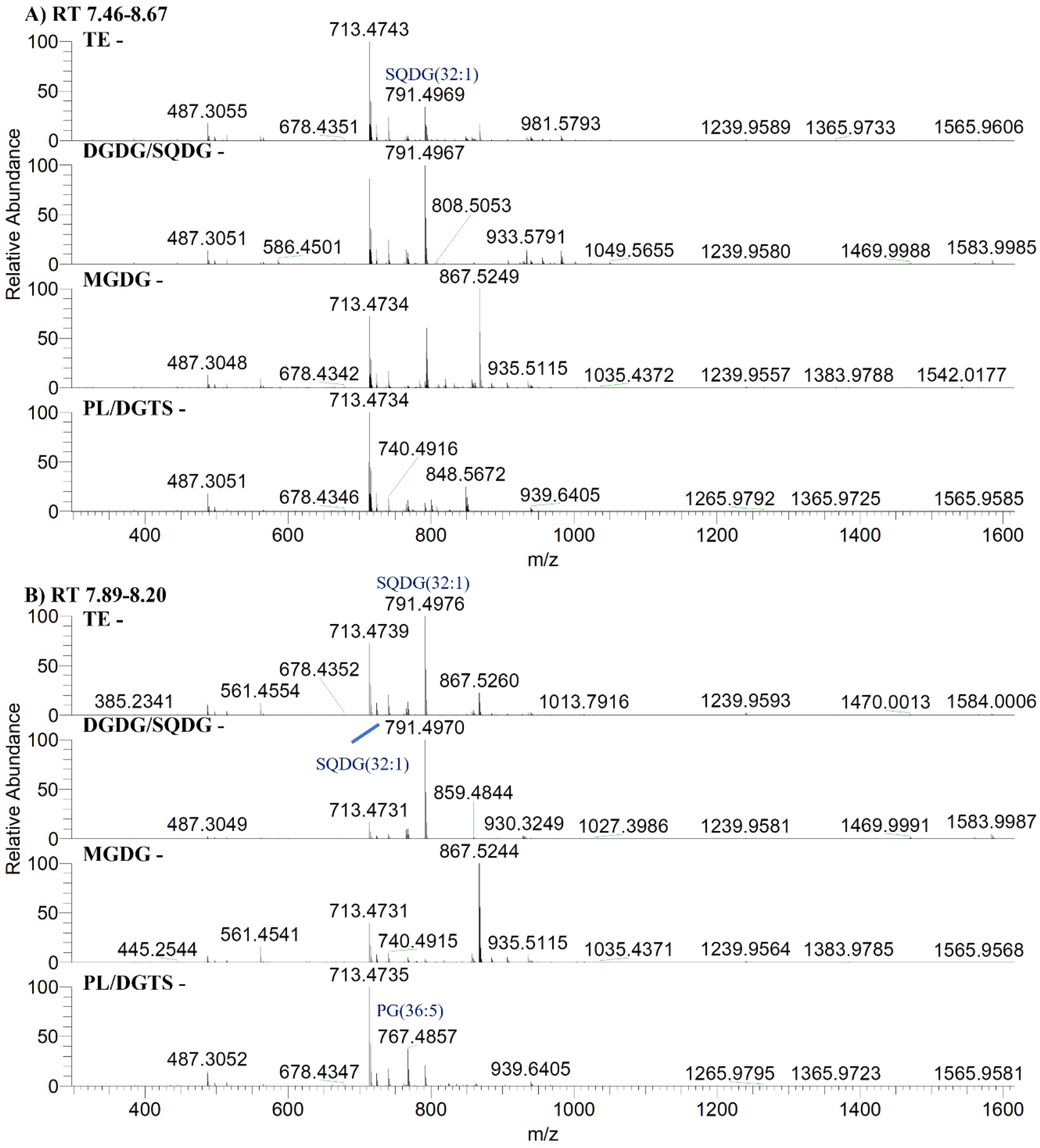


Figure S7.6. HILIC-ESI–MS spectra of the polar lipid species of *N. oceanica* identified in negative ion mode from elution time 7.46-8.67 (**A**) and 7.89-8.20 (**B**) in the total lipid extract and fractions (DGDG/SQDG; MGDG; PL/DGTS), after solid phase extraction.