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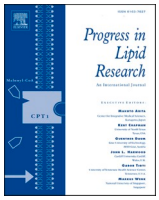

Review Article

Plant and algal lipidomes: Analysis, composition, and their societal significance

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ABSTRACT

Plants and algae play a crucial role in the earth's ecosystems. Through photosynthesis they convert light energy into chemical energy, capture CO2 and produce oxygen and energy-rich organic compounds. Photosynthetic organisms are primary producers and synthesize the essential omega 3 and omega 6 fatty acids. They have also unique and highly diverse complex lipids, such as glycolipids, phospholipids, triglycerides, sphingolipids and phytosterols, with nutritional and health benefits. Plant and algal lipids are useful in food, feed, nutraceutical, cosmeceutical and pharmaceutical industries but also for green chemistry and bioenergy. The analysis of plant and algal lipidomes represents a significant challenge due to the intricate and diverse nature of their composition, as well as their plasticity under changing environmental conditions. Optimization of analytical tools is crucial for an in-depth exploration of the lipidome of plants and algae. This review highlights how lipidomics analytical tools can be used to establish a complete mapping of plant and algal lipidomes. Acquiring this knowledge will pave the way for the use of plants and algae as sources of tailored lipids for both industrial and

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environmental applications. This aligns with the main challenges for society, upholding the natural resources of our planet and respecting their limits.

1. Introduction

Photosynthetic organisms such as higher plants, microalgae, and seaweeds (i.e., macroalgae) are a rich source of a complex variety of lipids. Due to their evolutionary history (for a review see [1]), plants and algae contain a wide diversity of lipids including phospholipids (PLs), neutral lipids (di- and triglycerides), and specific classes of lipids such as glycoglycerolipids, glycosyl inositol phosphoryl ceramides (GIPCs), betaine lipids (BLs) and phytosterols, that are commonly absent in most non-photosynthetic organisms. The glycerolipids (PLs, glyceroglycolipids, BLs) are enriched in omega-3 (ω-3) and omega-6 (ω-6) fatty acids (FAs), and thus these molecules have attracted interest of several sectors such as food and animal feed industries, health, green chemistry and energy sectors. The global availability of plants and algae, their enormous biodiversity, high productivity and ability to consume CO_2 are driving the research into understanding and analysing the lipid metabolism of these organisms.

The analysis of lipids in plants and algae is a challenge due to the complexity and diversity of the composition of the lipids and their modifications (e.g. hydrolysis or oxidation), and also because lipid composition seems to be quite specific for plants and algae [2]. Additionally, lipid composition is sensitive to the effects of environmental changes, which drive a significant adaptation and dynamic remodeling of the corresponding lipidome. Biotic and abiotic stress factors such as fluctuations of temperature, salinity, light intensity, exposure to heavy metals, eutrophication and their combination determine the content and composition of plant and algal lipids [3–8]. Changes in lipid classes, in particular their fatty acid composition, are currently an attractive direction in the quest for the specific biomarkers of ecological adaptations but can also be of interest for the production of biomass enriched in certain added value lipids for targeted applications. Therefore, to decipher plant and algal lipid metabolism (for a review see [9]) and composition, it is important to further develop analytical tools that allow the study and full coverage of the lipidome in these organisms.

Due to the high variability in the lipid composition of different species of plants and algae, significant efforts have been made to develop analytical procedures for efficient extraction of their lipids and analysis of their lipidomes. In the current era of Omics, lipidomics aims to target the knowledge related to lipids by covering the large-scale study of the structure and function of lipids and their interaction with other lipid and non-lipid molecules in biological samples, as well as unraveling lipid changes, namely the response to alterations in biotic and abiotic conditions [10,11]. Nowadays, lipidomics approaches using mass spectrometry technologies are the methodologies most used in the profiling and identification of lipid signatures. A new set of innovative mass spectrometry methods will facilitate the identification of the specific lipid species by addressing significant challenges related to complex chemical structures and low detection limits. Additionally, considering the screening methods needed for industrial applications, a tailored methodology, that can provide accurate results with short preparation time and low trial costs, will significantly advance the field.

Total lipid extracts or specific lipids isolated either from terrestrial plants, marine plants and algae are promising compounds for a wide spectrum of industrial uses. They are recognized as valuable phytochemicals for a multitude of applications in e.g., food, feed, nutraceutics, pharmaceutical, and cosmeceutical industries as well as the energy sector as a potential feedstock for biofuels. Thus, the identification of the lipidome of marine plants and algae (micro- and macroalgae) is essential to enhance the valorization of these compounds and foster innovative plant and algal-based solutions for food, biotechnological and industrial applications. Additionally, a critical point for algal lipid production is to select algal species with suitable lipid composition and yield. Therefore, to identify the best species/strains and culture conditions, analytical and detection techniques suitable for rigorous lipidomic analysis are urgently needed.

This review provides an overview of the different lipids present in plants and algae with the most recent extraction and lipidomic strategies. The potential use of these lipids and their fatty acids as important nutrients, biomarkers of ecological and climatic adaptations, and the future perspective for industrial applications are widely discussed underlying the particularities of plant and algal lipids, as an appealing source of smart omega lipids.

2. Plant and algae lipids: structural diversity and functions

The lipids identified in plants and algae comprise a diversity of different lipid classes, ranging from the basic units, such as FAs and free sterols to more complex ones, like phospholipids (PLs), triglycerides (TGs), betaine lipids (BLs) and glycolipids. While some lipids are shared between animals and plants/algae, like TGs, sphingolipids (SPs) or PL, others can be found mainly in algae, as BLs, or in photosynthetic organisms, as glycoglycerolipids, which can be found in chloroplasts/complex plastids. In the next sections, the main classes of lipids found in plants and algae are presented, and their chemical structure, biosynthesis, biological relevance and main methods of analysis are described.

2.1. Fatty acids

2.1.1. Classical fatty acids

FAs are carboxylic acids with acyl chains of different lengths, mainly with an even number of carbon atoms, between 12 and 24 carbons, but other chain length or odd numbers of carbons can be found, but in minor abundance. By convention, from 14 to 18 carbons, FA are called long chain fatty acid (LCFA) and above 20 carbons, they are called very long chain fatty acid (VLCFA), because in plant LCFA are synthesized in the plastid, whereas VLCFA are elongated from LCFA in the endoplasmic reticulum (ER) [12]. FAs can be saturated (SFA), or with one (monounsaturated FA, MUFA) to several double bonds (n = 2–6) (polyunsaturated FA, PUFA). The most common PUFAs are further classified into three types, the omega-3 (ω-3), omega-6 (ω-6) and omega-9 (ω-9) FA, depending on the location of double bonds in the carbon chain [13] (Fig. 1).

FA are the basic components of membrane lipids such as the PL, glycolipid and SP, of neutral lipids as TG and wax esters (WE), and of extracellular lipid polyesters such as cutin, suberin and sporopollenin, but the composition of FA within these lipid categories differs. In both algae and plants, FA play an important role in membrane structure and function, as they are involved in the fluidity of the lipid bilayer, its flexibility and selective permeability [16,17]. They provide energy to fuel the metabolic processes of the cell and act as signal transduction mediators [18].

The synthesis of long chain (14 to 18 carbons) FAs in algae and plants occurs in the plastids and cytosol and is mediated by the type II fatty acid synthase complex (FAS) [19]. FA synthesis typically starts from acetyl coenzyme A (CoA) and its carboxylation to malonyl-CoA extends the carbon chain. On the other hand, the polyketide synthase (PKS), localized in the cytosol, synthesizes either polyketides from acetyl-CoA in plants or some very long chain (VLC) PUFAs (as FA 20:5 or 22:6) in algae such as dinoflagellates [20]. In plants, VLCFA (from 20 up to 38 carbon atoms) result from the activity of the endoplasmic reticulum (ER)-associated multi-enzymatic fatty acid elongase (FAE) complexes [21]. FA desaturation generally produces cis double bonds and occurs in both

plastid and endoplasmic reticulum (ER), whereas elongation to VLCFA mainly happens in the ER. Both activities are highly influenced by environmental conditions [16,22]. However, one specific desaturation producing trans double bonds occurs in eukaryotic photosynthetic organisms on 16:0 in the *sn-2* position of the phosphatidylglycerol in chloroplast to produce 16:1Δ^{3t} [23]. In plant and algae, fatty acid can also be elongated in the ER from acyl-CoA exported from the chloroplast by the fatty acid elongase (FAE) complex [21,24]. Overall, fatty acid diversity is wider than what is described in this review and depends on the organisms and the environment [25].

The glycerolipids of algae and plants contain esterified SFA, MUFA or PUFA [18,19]. As an overall observation, marine algae, similarly to marine organisms are generally rich in VLC (above 20 carbons) PUFA, whereas freshwater algae and plants are rich in long chain (16 and 18 carbons) PUFA [9]. For instance, the freshwater green microalgae *Chlamydomonas reinhardtii*, *Chlorella vulgaris* and *Scenedesmus obliquus* contain SFAs with chain lengths from 14 to 18, mono-, di-, and poly-unsaturated FAs [16], while marine microalgae are generally rich in VLC-PUFA [9]. As an example, the diatom *Phaeodactylum tricornutum* is rich in FA 16:0, 16:1 and 20:5 [14] whereas the macroalga *Sargassum oligocystum* is rich in FA 16:0, 18:1 and 20:4, with a high proportion of SFA [28]. In land plants the FA that are esterified to glycerolipids (GLs) usually have a high amount of the essential ω-3 (FA 18:3, ALA) and are lacking VLC ω-3 PUFA (FA 20:5 and 22:6). FA are mainly present in esterified form; only a small amount of FAs are present in their free form (free FAs) in plants and algae due to their toxicity [29]. A high content of free FA can be found in some cases after harvest unless lipases are inactivated by heat treatment [30].

FA are usually analyzed by gas chromatography (GC) coupled with flame-ionization detector (GC-FID) or with mass spectrometry (GC–MS) after derivatization. The FA should be derivatized to obtain their methyl esters before GC analysis, namely by transmethylation in alkaline conditions or methylation in acidic conditions [31].

2.1.2. Unusual fatty acids

In addition to the above classical FAs found in plants and algae, an extreme diversity of unusual FAs, i.e. FAs with additional modification such as methyl branches, trans double bonds, conjugated (i.e. non-methylene interrupted) double bonds, acetylenic triple bonds, oxidations (presence of a hydroxy, keto or epoxy group) or cyclic structures (like cyclopropane or furan), has been described in plants and algae. For example, more than 450 different FA structures have been found in the storage lipids of seeds or other non-membrane lipids of vascular plants [25]. Many of these unusual FAs have a very high-value for specific industrial applications, thus numerous research projects have been devoted to the engineering of their production in common oilseed crops (reviewed in [32]). These intense research efforts have led to the elucidation of several very peculiar biosynthetic pathways which cannot be described in the present review (see [33] for details). Like classical FA, unusual FA are best characterized using GC.

2.1.3. Hydroxy fatty acids

Hydroxy FAs are characterized by a hydroxyl group in the acyl chain and can be found esterified to different lipids. Although they are often referred as to unusual FAs, some of them are ubiquitously found, and hydroxylation of the acyl chain can define some degree of specificity to certain lipid classes. In plants, the membrane glycerolipids usually do not contain a specific FA hydroxylation signature [34]. In contrast, membrane SPs contain a specific hydroxylation signature on the second carbon of the acyl-chain. This hydroxylation produces α-hydroxy FA (referred to as hFA), also called 2-hydroxy-fatty acids (referred to as 2OH-FA). In *Arabidopsis thaliana*, hFA can represent up to around 90% of the total SP pool [34,35]. Importantly, the hFA signature is not found in other pools of membrane lipids. The fatty acid hydroxylases (FAH1 and FAH2) and the cytochrome *b*5 (Cb5s) are required for efficient α-hydroxylation with FAH1 being more specific to VLC-FA while FAH2 selectively hydroxylates palmitic acid [35,36]. As a note, hVLCFAs are predominant in the pool of SPs with up to 30 carbon atoms [37]. At the functional level, it was shown that α-hydroxylation is important for stress response in *Arabidopsis* and immunity in rice [35,36,38]. The α-hydroxylation is known to be important for the interaction of SPs with sterols and the formation of ordered microdomains within the plasma membrane [39,40].

Besides the presence in membrane lipids, hydroxy FA accumulate in oils of some plant species. For example, in the seed oil of the *Euphorbiaceae castor*, about 90% of FAs in the TG pool are the hydroxy FA ricinoleic acid [41]. These FAs are usually synthesized by a divergent FAD2 desaturase in which a few amino acid substitutions are responsible for hydroxylation instead of desaturation [42].

In terrestrial plants, another predominant group of non-membrane

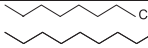
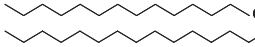
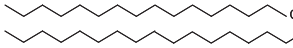
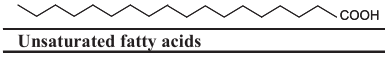
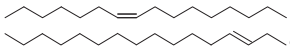
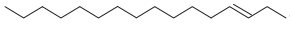
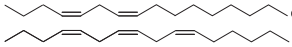
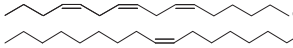
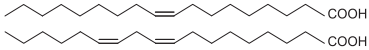
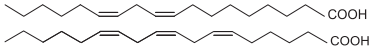
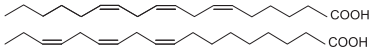
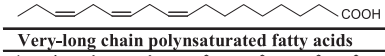
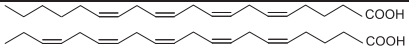
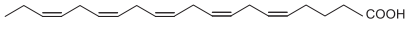
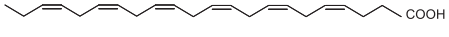
	Systematic name		Trivial name	Abbreviation
Saturated fatty acids				
	octanoic	8:0	caprylic	8:0
	tetradecanoic	14:0	myristic	14:0
	hexadecanoic	16:0	palmitic	16:0
	octadecanoic	18:0	stearic	18:0
Unsaturated fatty acids				
	*cis*-9-hexadecenoic	16:1$^{\Delta 9}$	palmitoleic	16:1ω7
	*trans*-3-hexadecenoic	16:1$^{\Delta 3t}$	palmitoleic trans-isomer* (in chloroplast phosphatidylglycerol)	16:1t
	*all-cis*-9,12-hexadecadienoic	16:2$^{\Delta 9,12}$		16:2
	*all-cis*-6,9,12-hexadecatrienoic	16:3$^{\Delta 6,9,12}$		16:3
	*cis*-9-octadecenoic	18:1$^{\Delta 9}$	oleic	18:1
	*all-cis*-9,12-octadécadiénoïque	18:2$^{\Delta 9,12}$	linoleic	18:2
	*all-cis*-6,9,12-octadecatrienoic	18:3$^{\Delta 6,9,12}$	γ–linolenic	18:3ω6
	*all-cis*-9,12,15-octadecatrienoic	18:3$^{\Delta 9,12,15}$	α–linolenic (ALA)	18:3ω3
Very-long chain polynsaturated fatty acids				
	*all-cis*-5,8,11,14-eicosatetraenoic	20:4$^{\Delta 5,8,11,14}$	arachidonic (ARA)	20:4ω6
	*all-cis*-5,8,11,14,17-eicosapentaenoic	20:5$^{\Delta 5,8,11,14,17}$	eicosapentaenoic (EPA)	20:5ω3
	*all-cis*-4,7,10,13,16,19-docosahexaenoic	22:6$^{\Delta 4,7,10,13,16,19}$	docosahexaenoic (DHA)	22:6ω3

Fig. 1. Main classical fatty acid in plants and algae. This list is not exhaustive and there are many other desaturated fatty acids such as 16:4 in *Chlamydomonas reinhardtii*, 18:5 in Ostreococcus tauri or in dinoflagellate and longer saturated fatty acids such as 24:0 in *Phaeodactylum tricornutum* [9,14,15]. Plant fatty acid diversity is well detailed in the plant fatty acid database https://plantfadb.org/.

lipids that contain a noticeable hydroxylation signature are extracellular lipid barriers, and more particularly the cutin, suberin and sporopollenin polyesters. The presence of high levels of ω-hydroxy fatty acids (hydroxylation at the terminal carbon, referred to as ωFA), α, ω-dicarboxylic fatty acids (presence of two carboxyl groups, referred to as DCA compounds), and sometimes of polyhydroxy-fatty acids allows the carboxyester-linkage of these monomers and the reticulation of the polyesters [43]. In comparison to the cutin polyester, suberin contains usually substantially more ω-hydroxy VLC-FAs [44]. The enzymes producing these hydroxy FAs are part of the cytochrome P450 family. In *Arabidopsis*, CYP86A1 and CYP86A8 ω-hydroxylates unsaturated C16-C18 FAs while CYP86B1 ω-hydroxylates VLC-FAs [45–47]. In-chain hydroxylation is catalyzed by members of the CYP77 subfamily, such as CYP77A6 which is involved in the production of 10,16-dihydroxy palmitic acid in *Arabidopsis* flowers [48].

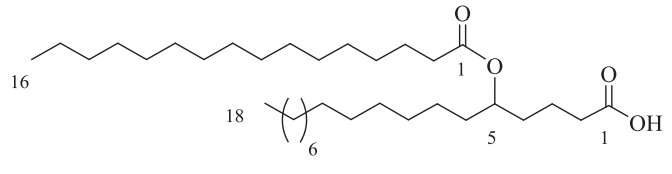
Finally, hydroxyl FAs are also found in lipokines, which are branched fatty acid ester of hydroxy fatty acids (FAHFA) (Fig. 2). These bioactive lipids discovered in 2014 by Yore and collaborators in mammals [49], and shown to exhibit anti-diabetic and anti-inflammatory effects. More recently, they were shown to be possibly involved in cancer [50]. FAHFAs have been qualitatively and quantitatively detected in cereals, vegetables and fruits and recently in fermented brown rice and rice bran with *Aspergillus oryzae* [51]. The quantification of FAHFAs is performed by LC-MS in the negative ion mode, as $[M\text{-}H]^-$ ions, due to the easy deprotonation of carboxyl groups [52].

To conclude, the type of hydroxylation (α,ω or in-chain) combined with the length of the acyl-chain could serve as a signature or a hallmark to channel different types of FAs to distinct lipid pools such as SPs, cutin or suberin. Targeting a substrate towards a specific pathway is a very attractive idea to explain how plants coordinate the spatial distribution of different lipid classes within a tissue or in a developing new organ, such as during the lateral root formation.

Hydroxy FAs are highly sought as chemical feedstock for industrial applications (for the production of polymers, plasticizers, surfactants or detergents, …). In particular, estolides (i.e. oligomeric fatty acid esters) represent very promising bio-lubricants for the oil and in cosmetic industries (reviewed in [53]). They are usually analyzed by GC–MS. As for FAs, they should be derivatized to obtain methyl esters and trimethylsilyl derivatized to improve thermal and hydrolytic stability and GC separation [34].

2.1.4. Fatty acid derived waxes

FAs are also found in the cuticular waxes covering the epidermal cells of the aerial parts of all land plants, either as minor components, as in *Arabidopsis*, or as major components, as in sorghum. Cuticular waxes are complex mixtures of VLC aliphatic components, all derived from saturated, unsaturated and branched VLCFAs. In the ER of epidermal cells, long-chain FA are elongated by the FAE complexes (up to 38 carbon atoms) and converted into aldehydes, alkanes, secondary alcohols and ketones through the alkane-forming pathway, or to primary alcohols and wax esters through the alcohol-forming pathway (reviewed in [54]). Wax components are then transported in a directional manner from the ER to the plasma membrane before being secreted to cover the cell wall of epidermal cells facing the external environment. Since each class contains various chain-lengths, these complex mixtures are best analyzed after sialylation by GC-FID or GC–MS.



5-PAHSA

Fig. 2. Example of a FAHFA with the chemical structure of the 16:0-(5-O-18:0) named the 5-PAHSA discovered in rice [51].

2.2. Complex lipids

2.2.1. Glycerolipids

Complex glycerolipids (GLs), widely distributed in higher plants and algae, belong to different categories: membrane lipids (PLs, glycolipids and BL) and TG that are present in lipid droplets. The glycolipids are present only in photosynthetic organisms and BLs are present in algae, bryophytes and some fungi and are absent in seed plants. Glycerolipids share a common structural feature of a glycerol backbone esterified to 1 or 2 fatty acyl chains at *sn-1* and *sn-2* position and linked at *sn-3* position to a polar head group for membrane lipids or another FA for TGs (Fig. 3).

2.2.1.1. Glycerophospholipids. The PL category, commonly referred to as glycerophospholipids, has a polar head linked to the *sn-3* position of the glycerol with a phosphate group and a polar moiety linked to the phosphate group. This polar group is specific of each PL class, defining the different classes of PL, like phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI) and cardiolipin (CL), or phosphatidic acid (PA) in the case of non-substituted phosphate (Fig. 4) [55]. The lyso forms of each PL class, corresponding to the PL esterified with only one FA, are also found (e.g., LPC, LPE). PL are synthesized in the ER, however PG is also synthesized in the chloroplast [9,56,57]. They are mainly carriers of PUFAs in plants and especially in algae, where they are greatly esterified with ω-3 PUFAs [58–60]. SFAs and MUFAs are also found in PL lipid species but in lower quantity. PL are the main building blocks of extraplastidial cell membranes and act as key signaling molecules. They are important to maintain cell integrity and function, and regulate several aspects of plant and algae development, defense against external stressful conditions and adaptation to biotic and abiotic factors. For example, plasticity of the lipidome of cell membrane is important to cope with alteration of environmental conditions, as temperature, salinity or nutrient stress among other stressors [61,62].

2.2.1.2. Glycoglycerolipids. In the glycolipid category, a glycosidic polar head group is linked to the *sn-3* position of the glycerol. There are three main classes of glycolipids in higher plants and algae (Fig. 5), depending on the sugar moiety and include neutral glycolipids, the galactolipids mono- and digalactosyldiacylglycerol (MGDG and DGDG, respectively) as predominant one, and an acidic, anionic glycolipid class, the sulfolipid sulfoquinovosyldiacylglycerol (SQDG). Oligogalactolipids (mainly tri or tetraGDG) can also be present in plants and algae with two routes of synthesis: successive galactosylation by DGDG synthase (DGD) or transgalactosylation from MGDG by the GL:GL galactosyltransferase (GGGT/SFR2). While the first route appeared early in the evolution (cyanobacteria), the second evolved associated to the process of terrestrialization in the streptophytes [63]. Another glycolipid class with a glucuronic acid-containing head group, the glucuronosyldiacylglycerol (GlcADG), has been reported but with low abundance and has been associated with response to phosphate starvation [64]. The lyso forms of glycolipids (i.e., MGMG, DGMG and SQMG) have been detected, but with lower abundance compared with the diacyl forms. In plants, GL are synthesized in the chloroplasts via so-called prokaryotic and eukaryotic biosynthetic pathways [9,56,57]. While in the prokaryotic pathway, all the reaction steps take place within the chloroplast (the plastidial pathway), there is a cross talk between the ER and the chloroplast in the eukaryotic (extraplastidial) biosynthetic pathway. Lipid migration between these organelles can occur, with transfer of some PL from the ER into the chloroplast with a key role as precursors of GL [57]. The eukaryotic and prokaryotic pathways in algae are more complex to follow due to less substrate specificity of the ER lysophosphatidic acid acyltransferase [65].

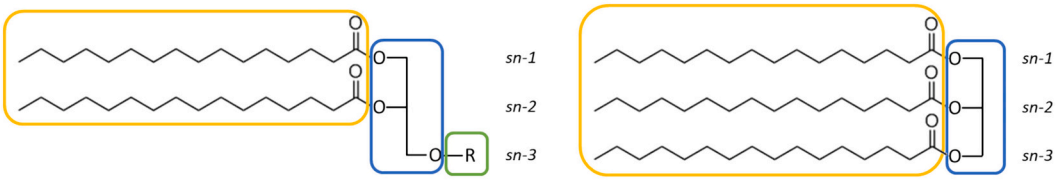


Fig. 3. Glycerolipid structures. Membrane glycerolipids including phospholipids, glycolipids and betaine lipids) (on the left) consist of a glycerol backbone (in blue) with two fatty acids (in yellow) esterified on the sn-1 and sn-2 positions and a variable polar head R (in green) on the sn-3 position. The triacylglycerides, often present in lipid droplets (on the right), contain three fatty acids esterified on the glycerol backbone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

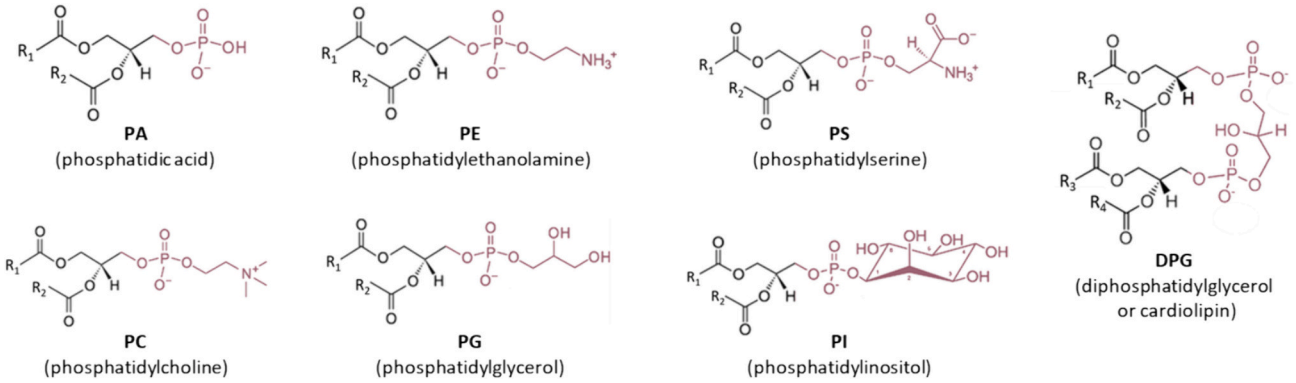


Fig. 4. Main structures of the phospholipids found in photosynthetic organisms. The glycerol backbone as well as radicals R_1 and R_2 corresponding to the fatty acid in sn-1 and sn-2 position are in black. The polar head containing a phosphate is in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

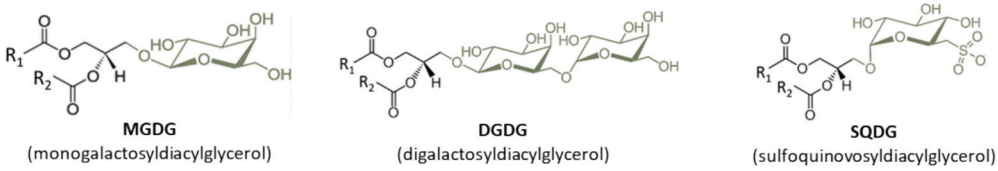


Fig. 5. Main structures of the glycolipids found in photosynthetic organisms. The glycerol backbone as well as radicals R_1 and R_2 corresponding to the fatty acid in sn-1 and sn-2 position are in black. The polar head containing a sugar is in colour.

The neutral glycolipids, MGDGs and DGDGs are mainly esterified with two PUFAs, while SQDGs contain commonly a SFA or MUFA and a PUFA [66]. Glycolipids are the main components of chloroplast membranes along with PG, although other minority PL are present on the outer envelope of plastidial membranes in plants [67]. The glycolipids and plastidial PG are also important to the assembly of the photosynthetic systems and thus have a crucial role in the photosynthesis process and on chloroplast function [68]. GL profile changes with nutrition limitation and with variation in light intensity and quality. For example, phosphate limitation was reported to increase GL such as the DGDG and SQDG [4]. In plants, a decrease in PL was observed under phosphate limitation and a translocation of DGDGs to extraplastidial membranes, which was suggested to contribute to balance the lack of PL in these membranes [14]. The plasticity of GL was also reported with different light intensities in macroalgae *Codium tomentosum* and *Bryopsis plumosa* (Bryopsidales, Chlorophyta), showing that high light-acclimation induced an accumulation of lyso-glycolipids [69,70]. In *Arabidopsis thaliana*, the ratio DGDG/MGDG is important to preserve membrane structure under temperature stress such as freezing [71] or heat stress [72].

2.2.1.3. Betaine lipids. BLs are a category of lipids with a betaine moiety linked by an ether bond to the *sn-3* position of the glycerol backbone. They are mainly found in algae and in lower plants, and not reported in seed plants [73]. There are three main classes of BLs (Fig. 6), 1,2-diacylglyceryl-(N,N,N trimethylhomoserine) (DGTS), 1,2-diacylglyceryl-3-O-2′-(hydroxymethyl)-(*N,N,N*-trimethyl)-β-alanine (DGTA) and 1,2-diacylglyceryl-3-O-carboxy-(hydroxymethyl)-choline (DGCC). Their lyso forms have also been detected. BLs are synthesized in the ER, DGTS by the reaction of DAG with S-adenosylmethionine [9], while DGTA, is supposed to be synthesized from DGTS [74]. BL are much less studied than PL and GL in plants and algae. They are mainly located in extraplastidial cell membranes and seem to replace PC in the case of phosphate limitation. Although quite unusual, BL were also found in chloroplast envelope membranes in a few algae, such as *Chlamydomonas reinhardtii* [61,75] and *Isochrysis galbana* [76], probably as a surrogate of PC found in the outer membrane of chloroplast envelope [77]. Specific BL classes can be found in evolutionary distinct algal clades [78]. In seaweeds, DGTS species were found in red (Rhodophyta) and green (Chlorophyta) seaweeds, while DGTA was found in brown (Ochrophyta) seaweeds in high abundance. Nevertheless, DGTS can be a minor class in algae from *Ochrophyta phylum* [79]. In the case of microalgae, a quite diversified distribution of BL was found in different species and phyla. For example, Cañavate et al. reported that the DGTS, DGTA and DGCC showed a dissimilar profile in several microalgae species and could be used as a chemotaxonomic tool [78].

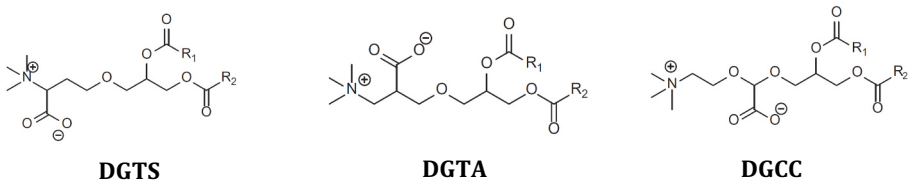


Fig. 6. Structure of the three known betaine lipids. DGTS: 1,2 diacylglyceryl-3-O-4′-(*N*,*N*,*N*-trimethyl)-homoserine, DGTA: 1,2-diacylglyceryl-3-O-2′-(*N*,*N*,*N*-trimethyl)-β-alanine, DGCC: 1,2-diacylglyceryl-3-O-carboxy-(hydroxymethyl)-choline). R_1 and R_2 correspond to the fatty acid esterified at the sn-1 and sn-2 positions, respectively.

2.2.1.4. Head group-acylated lipids. Head group-acylated lipids found in plants and algae include extra-plastidic head-group acylated lipids, such as *N*-acyl phosphatidylethanolamine (NAPE) and *N*-acylethanolamines (NAE), and every classical chloroplast lipids, namely the acylated MGDG, DGDG, SQDG and PG namely respectively acMGDG, acDGDG, acSQDG and acPG (Fig. 7) [80–82]. Although these lipid classes have already been identified in plants and/or algae, they remain to be systematically analyzed.

NAPE is a minor but ubiquitous class of membrane glycerophospholipids in plants. NAPE derived from direct acylation of phosphatidylethanolamine (PE), bearing a third FA attached to the ethanolamine polar head group through an amide bond [83,84]. The length and saturation degree of the three acyl chains greatly varies among NAPE molecular species. This triacylated PE was already identified in cereal grains (e.g., wheat, barley, oats), legumes, nuts, fruits, vegetables, and oils (e.g., hemp oil) [85] as well as in seeds like lupin seeds (*Lupinus luteus*) [86]. However, its synthesis can also be induced under stress conditions [87,88], with NAPE being the precursor for the formation of the lipid mediators NAEs after hydrolysis. NAEs were also identified in cereals, vegetables, nuts, oils (e.g., hemp oil, EVOO, palm oil) and legumes [85]. Despite being recognized as important structural and bioactive molecules, the role of these *N*-acyl lipids in plants is less characterized than in mammals. NAPE and NAE participate in plant growth and development processes such as seed germination, seedling establishment and growth, roots elongation, as well as in plant defense against pathogens as reviewed in [83,89].

Head group-acylated galactolipids have a third acyl group enzymatically esterified to the carbon at the 6′-hydroxyl group of the galactose. AcMGDG was detected in wheat (*Triticum aestivum*) and tomato (*Solanum lycopersicum*) leaves [90] as well as in red [91] and brown seaweeds [92], while acDGDG was detected in brown seaweeds [92], and acPG in oat seeds [93]. AcSQDG was detected in *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum* [14,82]. These head-group acylated lipids are formed as a common response under stress conditions [90,94]. For example, acMGDG was identified after heat stress [95], wounding, bacterial infection, and freezing [90]. The length and saturation degree of the three acyl chains vary between plant and algal species [90,96] and with biotic or abiotic stress [90]. Interestingly, oxylipin-containing acyl-MGDG and acyl-PG were found in *Arabidopsis thaliana* leaves after freeze-thawing [96] and hypersensitive response [94], respectively. The biological properties and roles of head group-acylated chloroplast lipids remain to be disclosed.

2.2.1.5. Triacylglycerols. TG is a family of glycerolipids that, unlike membrane glycerolipids, do not have a polar head at the *sn-3* position but a third esterified FA on the glycerol backbone (Fig. 3) [97]. TG molecules can therefore present symmetries (enantiomer) making it difficult to differentiate between the *sn-1* and *sn-3* position. The regioselectivity of TGs is a crucial point because the industrial properties of these high added value molecules differ according to the esterification position of the FAs [98]. In addition, the biophysical properties of TGs (high hydrophobicity, low polarity) make the accumulation of these

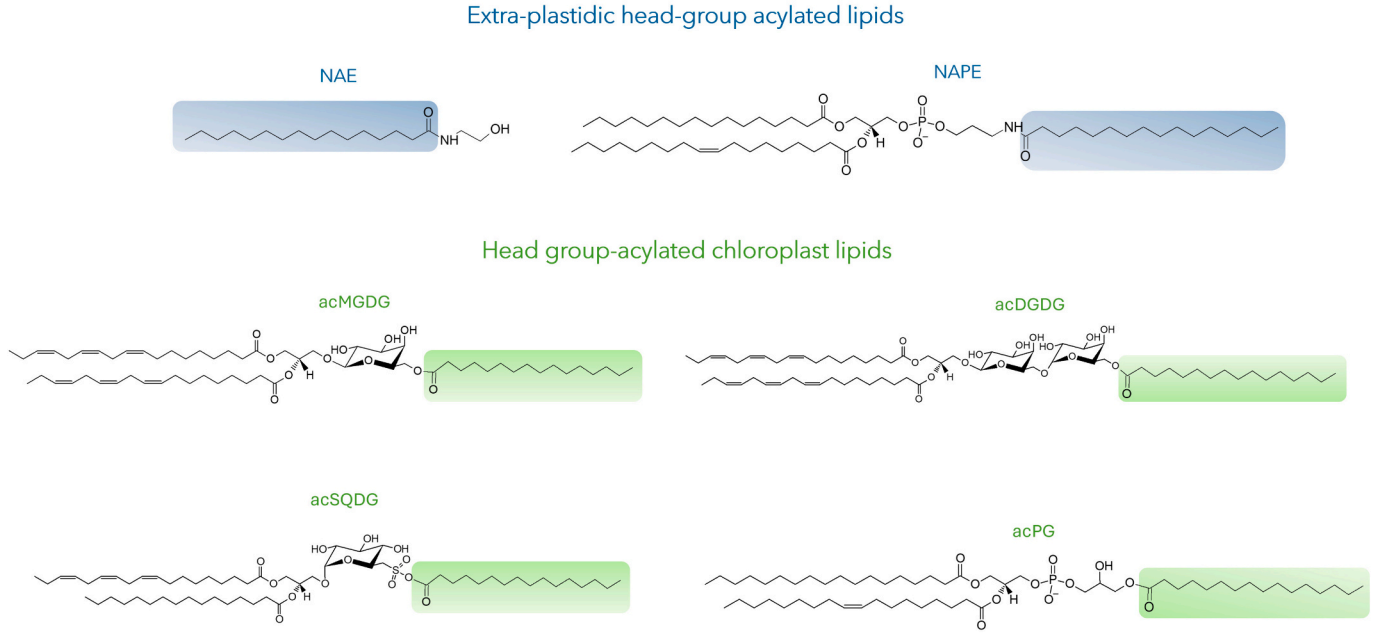


Fig. 7. Head group-acylated lipids found in plants and algae. NAPE, N-acyl phosphatidylethanolamine; NAE, N-acylethanolamine; acMGDG, acylated monogalactosyldiacylglycerol; acDGDG, acylated digalactosyldiacylglycerol; acSQDG, acylated sulfoquinovosyldiacylglycerol; acPG, acylated phosphatidylglycerol.

molecules difficult in biological membranes. They are synthesized mainly in the ER and stored in lipid droplets [99] but some TGs have been reported in the plastid and stored in plastoglobules [100]. However, TGs accumulate preferentially in cytosolic lipid droplets, budding from the outer leaflet of the ER in yeast and mammals [101] or the outermost membrane of the plastid in several microalgae species [102–104], with other neutral lipophilic components (e.g., sterols, pigments, etc.) when environmental conditions become unfavorable (e.g., nutritional deficiency, quality and intensity of light, high temperature, etc.) [14,102,105,106]. Thus, oleaginous microalgae, such as the diatom *Phaeodactylum tricornutum* [14,104] or the Thraustochytrids (e.g., *Auranthiochytrium limacinum*) [107], accumulate a large quantity but also a wide qualitative range (SFA, MUFA or VLC-PUFA) of TGs under nutrient deficiency. The TAG accumulation has become a real cross-disciplinary research (fundamental and applied) hub passing from human health, food, feed and green chemistry (e.g. biodiesel production) [97,108].

The different lipid categories, such as PL, glycolipids, BL and TG, are characterized by a great chemical diversity, not only due to the different classes that can be found, but also since the same categories or classes can be present in different relative contents and with different FA profile, depending on the plant and alga species and also on the environmental conditions [26,62]. Although a phylum trend can be seen, as shown recently for seaweed and for microalgae, it seems that the polar lipidome is quite species-specific [79]. Furthermore, the lipid profile is quite dissimilar between different tissues of plants as shown for *Arabidopsis* [109] and as across life cycle stages, as shown for *Porphyra dioica* [110]. Therefore, complex lipids are nowadays profiled to target the species-specific lipidome using lipidomic approaches based on LC-MS analysis.

2.2.2. Sterols

Phytosterols (sterols, ST) are amphiphilic isoprenoid lipids that are built from simple C_5 isoprenic units condensed into the $C_{30}H_{50}O$ linear sterol precursor 2,3-oxidosqualene. These triterpenoids (sterols) are initially formed by the action of lanosterol or cycloartenol synthases, which then define lanostane and cycloartane scaffolds (Fig. 8A). Common structural features of sterols are a C3β-hydroxy group, a tetracyclic (1,2-cyclopentanoperhydrophenanthrene) ring system and an aliphatic side chain at C17β. The structural diversity of the sterolome found in plants and algae is primarily caused by the number of unsaturation(s) in the tetracyclic ring (Δ^0 (stanols), Δ^5-, Δ^7-, or $\Delta^{5,7}$-sterols) and the type of side chain bearing 8 to 10 carbons and additional unsaturation(s) (Fig. 8B) [111,112]. Phytosterols are structural membrane components acting as reinforcers and regulators of membrane dynamics. As such, sterols exhibit strong actions on membrane associated biological processes. In addition, distinct functions in plant growth and development have been described for 24-methylsterols and 24-ethylsterols. The diversity and complexity of sterol profiles in plants and algae has also marked implications in the adaptation to environmental constraints [113,114].

Phytosterols are the precursors of a class of polyhydroxylated compounds called brassinosteroids which are well described in plants as major growth regulators (hormones). Phytosterols are also widespread in conjugated forms, the steryl conjugates that include the steryl glycosides (and acylated steryl glycosides) and the fatty acid steryl esters. Other conjugates like esters of ferulic acid are restricted to species from the Poaceae family [115,116]. Sterols esterified with FAs have an important role in cellular homeostasis [117].

Diversification of sterol pathways and profiles in plants and algae results in distinct types of 24-alkylsterols that differ by their side chain (C_8, C_9, C_{10}) and the stereochemistry at C24, which has phylogenetic implications [118]. 24-methyl- and 24-ethylsterols are found in all groups, 24α-alkylsterols are typical of higher plants whereas 24β-alkylsterols are usually found in algae. Likewise, isofucosterol is a plant sterol, whereas fucosterol is common in ochrophyte algae (e.g., Phaeophyceae and Bacillariophyceae). Sterols bearing a $\Delta^{5,7}$ diene system on the tetracyclic moiety are common in green algae (e.g., *Chlorella*, *Chlamydomonas*) but never found in plants, which display Δ^5 and/or Δ^7-sterols depending on the family/genera. Sometimes, similar sterols are produced in very diverging lineages due to evolutionary convergence, like with cholesterol being the most abundant sterol in multicellular red algae and being the backbone of specialized metabolites in Solanaceae [119]. Specialized sterol structures have been described in dinoflagellates, a group of endosymbionts that supply cnidarians hosts (sterol-auxotroph) with sterols [120]. C23-methylsterols and other so-called non-canonical sterols are mostly found in the oceans [121].

Sterol (ST) profiles from plants and algae are identified by GC methods after derivatization. GC–MS enables clear-cut identification of many structural sterols [111,122]. GC-FID is also a widespread method for sterol profiling and quantification. Steryl esters of FAs and steryl glycosides are analyzed by LC-MS methods with various ionization systems (electrospray ionization (ESI), atmospheric pressure photoionization (APPI), atmospheric pressure chemical ionization (APCI)) [113,123–125]. Classical thin layer chromatography (TLC) techniques allow the separation of free sterols and sterol conjugated forms, which

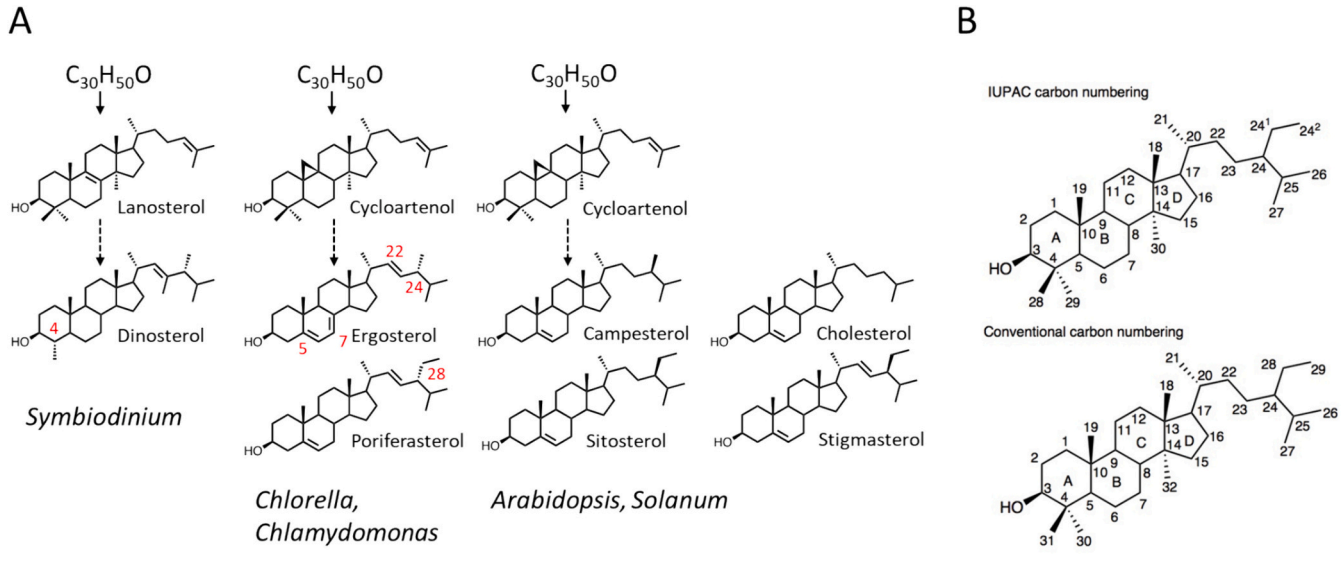


Fig. 8. Sterols in plants and algae. A, Diversification of sterol pathways and profiles in plants and algae – a simple view. B, Sterol structure nomenclature.

can be analyzed as sterol moieties by GC after deconjugation [126].

2.2.3. Sphingolipids

Sphingolipids (SLs) are a structurally diverse group of lipids containing hundreds of species with important roles in cellular membranes and biological processes. They are ubiquitous in all eukaryotic cells and are found also in some bacteria (for recent reviews of SL in plants, see: [37,127,128]. The basic structure of SLs is a hydrophobic amino alcohol carbon chain, known as a long-chain (sphingoid) base (LCB), containing at least two hydroxyl groups and an amine group. The LCB can be acylated to a FA through the amine group to form a ceramide, and ceramides can be further modified to more complex forms by the addition of polar headgroups, such as glycosyl and phosphate-containing groups (see Fig. 9). Plant SLs are commonly divided into four classes: LCBs, ceramides (Cer), glucosylceramides (GlcCers) and glycosyl inositol phosphoryl ceramides (GIPCs), which differ in their abundance in plant or algal cells by orders of magnitude [129].

Both the LCB and FA moieties can vary in their length and can undergo hydroxylation and/or desaturation. LCB length is usually between 16 and 20 carbons with up to four double bonds and possible hydroxylation at the C-4 position [130–132]. FA length is usually between 14 and 26 carbons with up to two double bonds and a possible hydroxylation at the C-2 position [132–135].

SLs are critical components of the plasma membrane but also part of the endomembrane system where they are synthesized, and some traces have been found in the tonoplast, mitochondria and the chloroplasts in response to stress [128].

SLs in plants are mainly composed of di- ('d') and trihydroxylated ('t') LCB with 18 carbons, with up to two double bonds at the Δ4 (trans) and Δ8 (cis/trans) positions. The FA component is mostly hydroxylated ('h') and can contain a double bond at the *n*-9 position [129,130]. GlcCers are commonly enriched with dihydroxylated LCB and C16 FA, while GIPCs are enriched in trihydroxylated LCB and longer FA (≥ C20) [134,136,137].

The study of SLs in algae is as extensive as that in terrestrial plants; nevertheless, studies have shown that algal SL are more structurally diverse, although usually less abundant than in higher plants [138]. The most studied SLs in both micro- and macroalgae are ceramides and GlcCers, while GIPCs are rarely reported, but usually identified in red algae [139–142]. Algal SLs are mainly composed of di- and trihydroxylated LCB with 18 carbons, with a possible branched methyl group at the C-9 position and up to four double bonds. The FA component can be hydroxylated and can contain up to two double bonds [131,132,141,143].

In plants, SLs are estimated to account for 30 to 40% of the plasma membrane lipids [136,144], of which the most abundant are the highly polar, anionic GIPCs and the GlcCers [129,144,145]. Thus, SLs are a critical component of the plant plasma membrane, affecting its fluidity and biophysical order. Together with sterols, SLs form ordered nanodomains, sometimes termed 'lipid rafts', which are involved in environmental sensing and stress response. As a major component of plasma membranes, SLs are significant in mitigating abiotic stress (e.g., thermal stress, salt stress, drought or hypoxia), both in plasma membrane remodeling and as signaling mediators [146,147]. Specifically, hydroxylation and desaturation of the FA and/or LCB moieties of the ceramide influence the thermodynamic properties of the membrane [148,149].

Plant SLs are also involved in multiple cellular, developmental and stress-response processes, including lipid bilayer fusion, cytokinesis, vesicle trafficking, plant development and defense [128,150]. Furthermore, they serve a regulatory role through the ceramides/LCB rheostat that regulates cell fate, where ceramides and LCBs are able to trigger cell death, while their phosphorylated form promotes cell survival or proliferation [151]. SL intermediates are involved in host-pathogen interactions, as part of the innate response mechanism [38,152]. Furthermore, some fungal and bacterial plant pathogens produce toxins that modulate SL metabolism. Specifically, during necrotrophic interactions, an increase in LCB abundance because of pathogenic mycotoxins induces cell death, while inhibition of LCB synthesis by biotrophic toxins prevents it.

The functional role of algal SL is largely understudied. Specific GlcCers were found to induce programmed cell death during viral infection of *Emiliania huxleyi* [143]. In addition, resistance and susceptibility to viruses were correlated with the presence or absence of

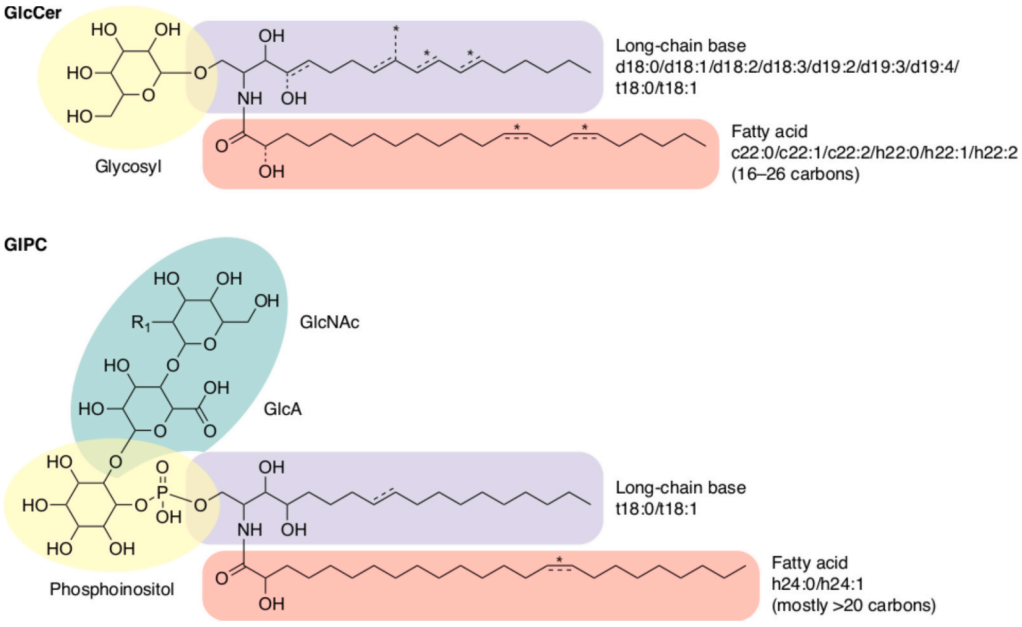


Fig. 9. Structure of glucosylceramides (GlcCers, top) and glycosyl inositolphosphoceramides (GIPCs, bottom) reported as plant and alga SLs. Dashed lines mark potential modifications of ceramide structure. * marks that the position of the double bond or methyl is not determined. Purple background indicates Long-chain bases (LCB), red background indicates fatty acyl chain, and yellow background indicates the headgroup. Green background indicates additional modifications of the headgroup. GlcNAc, N-Acetylglucosamine; GlcA, glucuronic acid. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

specific SL species [153–155], suggesting their involvement in conferring these phenotypes. However, further functional studies are warranted to better understand the role of SL in algal response to biotic and abiotic stress. SLs are usually analyzed by LC-MS, and are well detected in the positive ion mode MS analysis. Phosphate-containing SLs, like GIPCs, can be detected both in the positive and negative ion mode MS analysis. Other analytical methods include TLC or HPLC coupled with evaporative light-scattering detection (ELSD). HPLC coupled with UV or fluorescent detector can be used for LCB analysis, after deacylation (for complex SLs) and modification of the free amine by the addition of a chromophore (reviewed by [156]).

2.3. Epilipids: oxidized and nitrated lipids

2.3.1. Oxidized lipids

Several PUFAs, essential for living organisms, are present in land plants and algae, among them, α-linolenic acid (ALA, 18:3 *n*-3), an *ω*-3 PUFA. This PUFA is composed of eighteen carbon atoms and possesses three carbon-carbon double bonds. Other PUFAs have been only identified in marine algae, such as arachidonic acid (AA or ARA, 20:4(ω-6)). Their double bonds form skipped diene structures (two double bonds separated by a methylene group (-CH_2-) are prompted to peroxidation, which lead to the formation of oxygenated metabolites, named oxylipins [157,158].

The oxygenated lipid species of membrane complex lipids have been identified in plants and algae and seem to play a role in the adaptive mechanism to combat both abiotic and biotic stress. Oxidized (ox) PL (PC, PE, PG, PI, PS) and glycolipids (MGDG, DGDG) were identified in barley roots as a defense mechanism against salinity [159]. Some oxPL (PC, PE and PG), oxidized glycolipids (MGDG and SQDG) and BLs (DGTS and MGTS) have been identified in the lipidome of a few microalgae, such as *Chlorella vulgaris* [58], *Chlorococcum amblystomatis* [160], *Chrysotila pseudoroscoffensis* [161], *Cyanidioschyzon merolae* [162], *Galdieria sulphuraria* [163] and in brown, red and green macroalgae, such as *Laminaria digitata* [129], *Palmaria palmata* [79] and *Codium tomentosum* [164]. Even when molecules are similar to what exists in other organisms, their biosynthesis routes are partially distinct [165]. Oxidized lipid species of PC, LPC and SL classes have also been identified in olive seed oil [10]. However, their roles and biosynthesis are still unclear.

Actually, two biosynthetic routes are known, and in both radicals can easily abstract one hydrogen atom on the methylene of the PUFAs to produce a reactive species that will undergo further oxidative steps when reacting with molecular oxygen. Radicals may be an amino acid derived radical from enzyme active sites (e.g., tyrosyl radical in cyclooxygenase (COX) or dioxygenase (DOX) [166]), or smaller free radicals (e.g., $OH^{\bullet}$ coming from Fenton reaction). Once this first hydrogen abstracted, the radical peroxidation cascade will proceed whether in the active site of the enzymes, leading to enzymatic oxylipins, or in the lipid membrane, leading to non-enzymatic oxylipins. It should be mentioned that both pathways (enzymatic and non-enzymatic) might involve common intermediates such as fatty acid-hydroperoxides. To summarize, two main families of oxylipins might be formed depending on the mechanism involved, enzymatic (Fig. 10) or non-enzymatic (Fig. 11) ones.

Figure 10 represents a summary of the enzymatic biosynthetic pathways and highlights the main families of enzymatic oxylipins discovered until now. ALA is one of the main PUFAs in plants and algae and some oxylipins described in Fig. 10 are specific to plants and algae (underlined). Enzymatic derived oxylipins, such as LOX-derived oxylipins, are involved in various physiological processes and have important roles in the adaptation of plants and algae [167]. Several analytical methods have also identified the different types of oxylipins produced in plants [168], and thanks to chemical synthesis or biocatalysis, that have increased the quantities and numbers of oxylipins available, which made biological studies easier.

As for the PUFAs, oxylipins have been found in a myriad of plants as well as in various parts of the plant (leaves, roots) [169–171]. Regarding aquatic plants, fresh and marine algae particularly, oxylipins have been discovered in several brown, red, and green macroalgae [169,172,173] and some microalgae (diatoms) [174]. Jasmonic acid (JA) and its derivatives, among which the phytohormone jasmonoyl-isoleucine, are

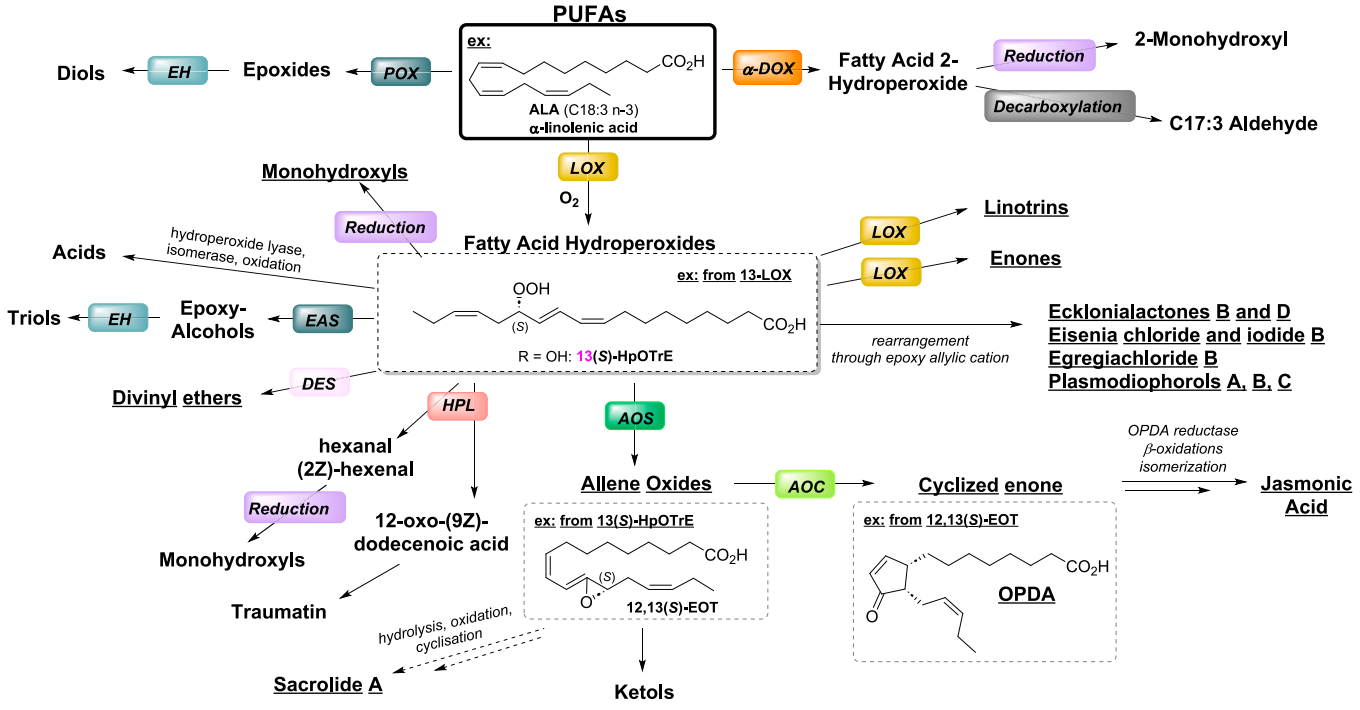


Fig. 10. Enzymatic pathways of PUFAs' oxidations and some examples of ALA-oxylipins. Underlined the main ALA-enzymatic oxylipins found in plants and algae. LOX = lipoxygenase, DOX = dioxygenase, POX = Peroxygenase, EAS = Epoxy Alcohol Synthase, EH = Epoxide Hydrolase, DES = Divinyl Ether Synthase, AOS = Allene Oxide Synthase, AOC = Allene Oxide Cyclase, HPL = HydroPeroxide Lyase, HI = Hexenal Isomerase.

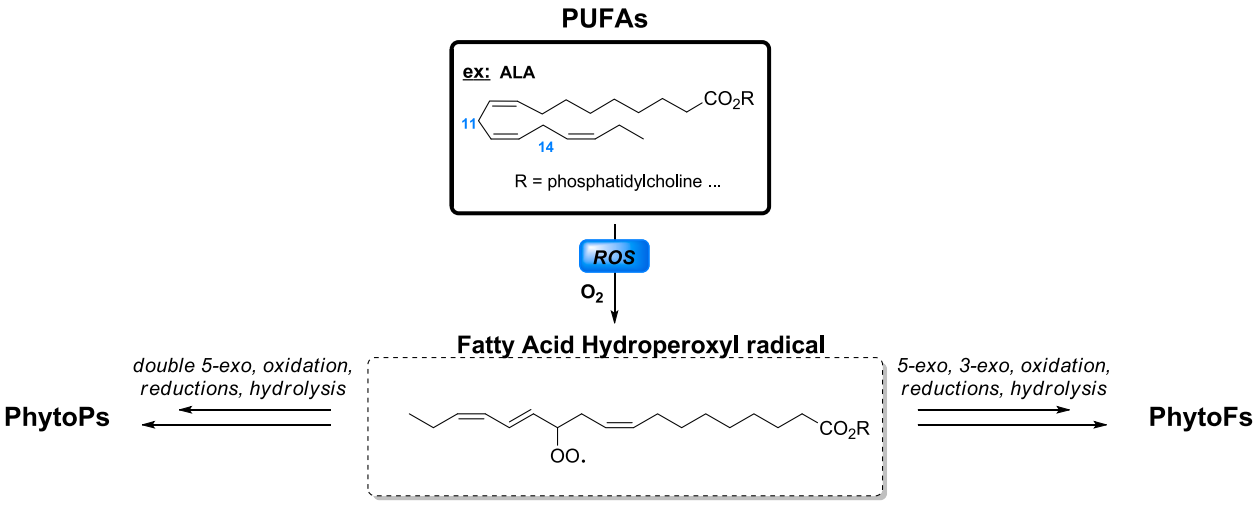


Fig. 11. Non-enzymatic pathways of PUFA oxidations.

important for plant response to pathogens are mainly found in seed plants [175]. While the signal transducing machinery seems conserved at least in bryophytes, the COI1 receptor is not bound by jasmonoyl-isoleucine, but by the JA precursor dinor-OPDA in the liverwort *Marchantia polymorpha* [176]. JA and/or methyl-jasmonate (Me-JA) were detected in some macroalgae, such as *Gelidium latifolium* [177] and in microalgae such as *Chlorella* [178] as well as *Dunaliella* [179]. The physiological role of those molecules is not proven, but other oxylipins, like derivatives of arachidonic acid, are clearly involved in induced chemical defences [180].

Phytoprostanes (PhytoPs) and phytofurans (PhytoFs) are C18 oxylipins, formed inside the membrane. However, there is evidence that enzymatic formation can also occur inside the lipid membrane [181]. If attached to the membrane such as in phospholipids or galactolipids, they are released by enzymes (e.g. phospholipases or acyl hydrolases).

Also, as described for the enzymatic oxylipins, ALA possesses labile hydrogens, leading through a radical cascade to oxylipins. For both PhytoPs and PhytoFs, the biosynthetic route starts with the abstraction of a hydrogen on one of the bisallylic positions (C11 or C14), that forms a pentadienyl radical which reacts with O_2. The resulting hydroperoxyl radical (Fig. 11) performs a 5-*exo*-trig cyclization leading to a 1,2-dioxolanylcarbinyl radical. A second 5-*exo*-trig cyclization produces a bicyclic endoperoxide, which, after another reaction with molecular oxygen, reduction and hydrolysis gives PhytoPs [157] On the other hand, from the 1,2-dioxolanylcarbinyl radical, a bisepoxide can be formed leading to the formation of PhytoFs after addition of water, reduction and hydrolysis. [182]

PhytoPs have been detected in various matrices. PhytoPs were highlighted in diverse food (e.g., beans, chocolate, and wine) and especially in nuts, seeds and vegetable oils [183]. In algae, PhytoPs were observed for the first time in 2015 by Barbosa et al. in 24 macroalgae species (3 Chlorophyta (green algae), 16 Phaeophyta (brown algae) and 5 Rhodophyta (red algae) [184]. In 2018, Lupette et al. studied the diatom *Phaeodactylum tricornutum* under oxidative stress [174], and despite the low concentration of ALA (2% of total fatty acids) in *Phaeodactylum tricornutum* cells, PhytoPs were the main non-enzymatic oxylipins in stressed cells.

In plants, Parchmann and Mueller have detected PhytoPs in four plant species belonging to Solanaceae, Fabaceae, Apocynaceae and Poaceae families [185]. Since then, they were found in pollen birch using GC–MS with derivatization and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) without derivatization where *ent*-16-B_{1t}-PhytoP turned out to be the most abundant [186,187]. In 2016, 8 PhytoPs were identified and quantified by Yonny et al. in melon leaves exposed to high temperatures [188]. They showed that levels of PhytoPs were from 1.6 to 2.2 times higher on stressed samples than in control samples. Very recently, they were also found in *Arabidopsis* under photo-oxidative stress conditions by Rac et al. [189]. The quantity of some PhytoPs considerably increased when the leaves were exposed to light.

As for PhytoPs, obtaining PhytoFs by extraction is difficult and chemical synthesis has been employed. To date, a unique strategy has been developed and three PhytoFs were obtained [190]. The synthetized *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}–9-PhytoF was further identified and quantified in seeds and nuts (e.g., pine, walnut, chia and flax), thanks to its obtention by chemical synthesis [191]. In addition, PhytoFs were also identified and quantified in melon leaves submitted to thermal stress, *Arabidopsis* and pollen birch [187–189]. Finally, a study by Vigor et al. focused on two red and four brown marine macroalgae reports the abundance of *ent*-16-(*RS*)-9-*epi*-ST-Δ^{14}–10-PhytoF under copper stress conditions compared to all isoprostanoids [192].

These oxylipins are currently mainly detected thanks to targeted lipidomic, using LC-MS/MS techniques [193]. However, immunological or GC–MS techniques were also developed [194–196].

2.3.2. *Nitrated lipids*

Nitro-fatty acids (NO_2-FAs) are well-known products of the reaction between free or esterified FA and reactive nitrogen species [197]. Free NO_2-FA were already reported in the lipidome of plants, but not yet in algae. The nitro derivative of linoleic acid (NO_2-LA) was identified in both cell-suspension cultures and seedlings of the model plant *Arabidopsis thaliana* [198,199], in the roots, leaves and subcellular (mitochondrial and peroxisomal) fractions from pea (*Pisum sativum*) [198], and in leaves from rice (*Oryza sativa*) plants [200]. Additionally, both the nitro derivative of conjugated linoleic acid (NO_2-cLA) and the nitro derivative of oleic acid (NO_2-OA) were found in extra-virgin olive oil (after TAG hydrolysis by pancreatic triacylglycerol lipase) and in fresh olives (protein cysteine adducts of NO_2-OA), respectively [201]. NO_2-OA was also found in seeds and differently developed seedlings of oilseed rape (*Brassica napus*) [202]. However, nitrated PL, that were already detected in mammals' cells and tissues, remain to be identified in both plants and algae. NO_2-FAs in plants have been identified by untargeted and targeted lipidomics approaches bases on LC-MS analysis.

NO_2-FAs play key signaling roles during plant growth and development but also in plant defense responses under biotic and abiotic stress conditions such as mechanical wounding, low temperature, and cadmium or salinity stress [199]. The physiological roles of NO_2-FA can be mediated by their ability to release nitric oxide (NO) or by electrophilic adduction to targeted proteins [203]. In fact, protein cysteine adducts of NO_2-OA were detected in fresh olives [201].

3. Analytical workflows for lipid analysis

The high diversity and complexity of plant and algal lipids leads to distinct lipid profiles, which vary greatly among organisms [79], with life cycle [110] and developmental stages [109], and with the growing environmental (e.g., geographic origin [204–207] or season of harvesting [10,208]) and culture conditions [58,209]. Therefore, the analysis of these complex chemical structures that can be detected in total lipid extracts of plants and algae and the estimation of their amounts requires the utilization of different but complementary analytical methods. The following section summarizes the workflows and the principal analytical techniques used for the identification and quantification of lipids from plants and algae.

Lipid analysis workflow (Fig. 12, Table 1) includes the following steps: collection/ harvesting and preservation, preparation of samples (including lipid extraction and fractionation), methods for lipid analysis, and data analysis and interpretation.

3.1. Harvesting and preservation

Plant and algal material possess very active lipases, mainly phospholipase D for plants producing phosphatidic acid (PA) [210] and phospholipase A1 for algae generating free fatty acid and lysolipid [211]. Therefore it is important to use an appropriate harvesting and storage process to avoid any degradation of lipids. For plants, significant accumulation of PA will be a sign of lipid degradation, whereas for algae it is indicated by an accumulation of free FA.

Wounding activates phospholipase D in plant within minutes [210]. To avoid phosphatidic acid accumulation in tissues after cutting, samples need to be frozen in liquid nitrogen very quickly and then stored at −80 °C. For microalgae, harvesting is a costly part of the process that involves dewatering. Different techniques are available such as coagulation and flocculation, flotation, centrifugation and filtration [212] and as for plants, to avoid lipid degradation and free FA accumulation, freezing the biomass in liquid nitrogen and storing it at −80 °C is advised. Another strategy would be to subject the fresh plants or algal material to thermal processing (10 min at 100 °C in a water bath) before storage at −20 °C, to limit lipolysis before extraction [213].

3.2. Extraction and fractionation

Due to the high variability in the lipid composition of the different species of plants and algae, significant efforts have been made to address analytical procedures for an efficient extraction of their lipids. For the extraction of lipids, there are two significant challenges to overcome: extraction efficiency with minimal lipid degradation, and removal of non-lipid contents.

Table 1
Analytical methods used for identification and quantification of the different lipid categories identified in plants and algae.

Plant and algal lipids	Analytical method
Fatty acids	GC–MS; GC-FID; FTIR
Hydroxy fatty acids	GC–MS; LC-MS; FTIR
Waxes	GC-FID and/or GC–MS
Suberin and cutin polyesters	GC–MS
Glycerolipids	TLC coupled to colorimetry, LC-MS, NMR; FTIR
Sterols	GC–MS; GC-FID; TLC; LC-MS; FTIR
Sphingolipids	LC-MS; FTIR
Oxidized lipids	GC–MS; LC-MS
Nitrated lipids	LC-MS

To avoid degradation, lipases activity is blocked during the extraction process by boiling the sample in alcohol or by addition of a secondary alcohol such as isopropanol [66,214]. Another strategy would be to carry out the entire extraction process under cold conditions [215]. For the extraction efficiency, a key challenge is the low recovery rates of lipids for some plants or algae, which has been attributed to the rigidity of algae or plants matrices, which delay the release of compounds. Additionally, the components present in the cell matrix have a significant influence on the efficiency and yield of lipid extraction. Sample pretreatment appears as a critical step in an effective extraction procedure [216]. Procedures involving lyophilization, inactivation of lipases, and methods for cell-disruption are described to affect largely the yield of extraction and the extractability of the different lipid classes in several plant and microalga species [217,218]. Recently other suitable pretreatment methods before extraction or the application of different combinations of novel techniques such as enzyme-assisted, microwave-assisted, ultrasound-assisted, supercritical fluid, and pressurized liquid extraction have been pointed to enhance the recovery of target lipids for industrial applications [219]. The influence of these pre-treatments depends largely on the solvent mixture employed, thus testing these effects is highly recommended.

Two strategies are adopted for the extraction method: either a global extraction or a targeted extraction for specific class analysis. For global extraction, the extraction efficiency needs to be as similar as possible for

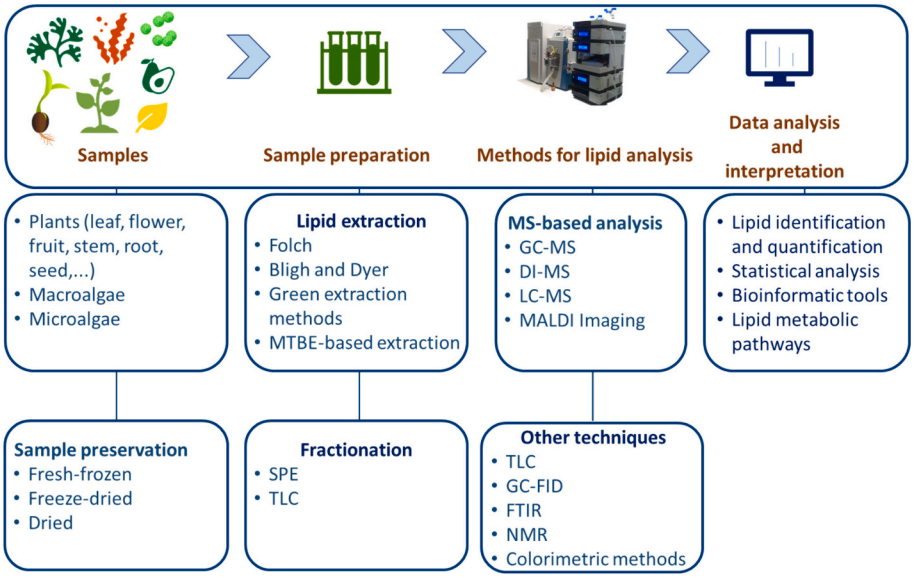


Fig. 12. Workflow summarizing the main steps for lipid analyses in plants and algae.

all the lipid classes that need to be analyzed. This aspect makes the standardization of the lipid extraction protocols a difficult task [220] due to the great heterogeneity of plant and algal lipids and cells. The standard methods commonly used are biphasic methods based on mixtures of different organic and alcoholic solvents (organic phase) and an aqueous phase. Classical methods use chloroform and methanol as organic solvent and are based on the Folch [221] and the Bligh and Dyer methods [222]. A similar method using MTBE (methyl-tert-butylether) was more recently developed with the advantage of having the organic phase above the aqueous phase instead of more classical methods with chloroform being under the water phase [223]. All these methods have been adapted for plants and algae, depending on the organism [218,224] (Fig. 12).

For more targeted purposes, mixtures with better extraction efficiency for neutral than polar lipids have been used with different performance depending on the alga or plant species, such as hexane/methanol, hexane/ethanol, hexane/isopropanol, and cyclohexane/1-butanol [224]. In some cases, extractions were performed by using Soxhlet, pressurized liquids, and supercritical extraction, but showing dissimilar extractability of the different lipid classes. Low polarity solvents are more proper for the extraction of neutral lipids while polar lipids, such as glycolipids and PL present in chloroplasts and other cellular membranes are more effectively extracted using more polar solvents, such as ethanol or methanol [224]. In recent years, direct extraction of microalgal biomass using non-toxic and environmentally friendly polar solvents such as ethanol or supercritical CO_2, as well as using hexane and acetone has been described as an efficient protocol to extract neutral and polar lipids together [58,225,226].

For specific lipid classes, such as GIPCs, oxidized and nitrated lipids, phosphatidylinositol phosphate, specialized methods have been developed. For example, GIPCs are insoluble in traditional lipid extraction solvents, such as chloroform/methanol and may be extracted using other methods through a monophasic procedure [39]. For hydroxy fatty acids, different protocols have been reported depending on the matrix [49–52]. For biological studies a classical Bligh & Dyer liquid-liquid extraction, under acidic conditions, using citric acid led to increase the yield of extraction. From rice powder, Watanabe et al. [227] used ultrapure water followed by methanol, then shook for 1 h and centrifuged. The organic phase was purified by liquid-liquid extraction and the upper ether phase containing the hydroxylated fatty acids evaporated to dryness.

The total lipid extracts can be further quantified by gravimetry and/or analyzed directly by MS or LC-MS. Nevertheless, total lipid extracts can be submitted to fractionation or enrichment steps aiming to obtain purified or enriched fractions of the different lipid classes using the solid phase extraction (SPE) technique [204,228,229] or thin layer chromatography (TLC) [230,231]. In SPE extraction, total lipid extract is fractionated in different lipid-rich fractions according to their polarity, such as fraction rich in neutral lipids, fraction rich in pigments, fraction rich in glycolipids and fraction rich in phospholipids and betaine lipids. Lipid extracts from plants or algae are usually fractionated using glass or plastic columns containing silica gel or aminopropyl as stationary phase (e.g., SPE—Si, NH_2-SPE cartridges), which can be used separately or sequentially [229]. Sequential elution solvent systems can be adapted to obtain fractions enriched in lipids or specific lipids. The column is conditioned with a solvent (e.g., *n*-hexane or heptane) to activate the stationary phase, prior to the application of total lipid extract. Afterwards, a sequential elution of different solvents is applied to obtain lipid fractions. For example, to fractionate algal lipid extracts, silica gel columns are used with the following sequential solvent system: chloroform to obtain the neutral lipid rich fraction, diethyl ether/acetic acid (98:2, *v/v*) for pigments, acetone/methanol (9:1, *v/v*) for fraction rich in glycolipids and methanol to obtain the fraction containing PLs and BLs [204,228,232,233].

TLC was one of the first separation methods introduced for lipid analysis and can be used for lipid class identification or fractionation. TLC is a versatile method, is easy to perform, and does not require expensive instrumentation. In this chromatographic technique, the stationary phase is spread as a thin layer on a plate of glass or aluminum foil whereas the mobile phase is a liquid which moves by capillarity along the plate. As a result, the analyzed sample is separated into spots/bands with different migration distance on the layer (measured as retention factor, Rf value) depending on the molecular structure of the analyte [31,234]. In the analysis of plant and alga glycerolipids, the most widely used TLC stationary phase is silica gel, with various solvent systems as a mobile phase depending on the polarity of studied lipids, e.g. hexane- or chloroform:methanol based mixtures for neutral or more polar lipid classes, respectively. The separation of lipids will be performed according to their affinity with the stationary or mobile phases [235]. Incorporation of silver ions in the layer (the so-called silver ion TLC, Ag-TLC) causes additional molecular interactions which ensure separation of lipid molecules depending on the number, configuration (cis/trans) or even isomeric positions of their double bonds. Ag-TLC has been applied for analysis mainly of fatty acids and TGs [236]. Likewise, silica gel (or Kieselguhr) layer coated with C18 chains can be used for reversed-phase TLC (RP-TLC) with mobile phase composed of polar solvents including water, methanol, acetonitrile, etc. [237]. Classical TLC techniques allow also the separation of free sterols and sterol conjugated forms [238]. TLC techniques can be applied in preparative or analytical modes [239,240]. On the other hand, analytical TLC is used for qualitative or quantitative analysis the latter performed by densitometry of charred lipid spots or by flame ionization detection in Iatroscan system [241]. Fully automated TLC, namely the high-performance TLC (HPTLC by CAMAG or BIONIS equipments) allows coupling even to mass spectrometry.

After extraction, with or without further purification by TLC or SPE, lipid extracts can be analyzed by different approaches, from simple methods such as colorimetry to much complex methods like mass spectrometry coupled to chromatography (e.g., GC–MS, LC-MS), but also with more global, non-destructive and less conventional methods such as FTIR, NMR and Raman microscopy.

3.3. Analytical methods used for the identification and quantification of lipids

3.3.1. Colorimetric quantitative methods

Colorimetric methods are used for the quantification of total lipids and specific lipid classes (total PL, GL) from samples (e.g., biomass, cells), lipid extracts and fractions. Although the total lipids amount is frequently estimated by gravimetry after total lipid extraction, colorimetric methods have been developed to reduce the time consumed for lipid extraction by performing lipid determination directly in the samples (e.g., freeze dried biomass, or oils), being the most common the sulfo-phospho-vanillin (SPV) assay [242,243]. This colorimetric method is based on the formation of a chromogen by initial reaction of lipids with concentrated sulfuric acid followed by generation of a pink chromophore after vanillin addition [244]. For example, the SPV assay was employed for the quantification of lipids in dried biomass of microalgae and vegetable oils, using calibration curves prepared with commercial oils and absorbance measurement at 530 nm [242].

The total content of PL in total extracts or in purified PL fractions, can be obtained using the Bartlett and Lewis method [245]. This method quantifies the organic phosphates released after acid hydrolysis of PL with perchloric acid and subsequently mixed with water and sodium molybdate to create a complex phosphate-molybdate. This procedure should be performed in a hood designed for perchloric acid use. After addition of ascorbic acid, the absorbance of the samples is measured at 797 nm. The amount of PL is estimated by multiplying the phosphate amount per the conversion factor 25, that would be the ratio between the average molecular weight of PL and phosphorus molecular weight [246]. Another method for determining PL without using perchloric acid is the phosphate assay described by Ames [247]. This method involves using a solution of magnesium nitrate in 95% alcohol. The mixture with

the sample is then taken to dryness by shaking the tube in flame. After adding HCl and heating to hydrolyze to phosphate, a mixture of ascorbic acid and ammonium molybdate is added. The absorbance is then measured at 820 nm for determination of total phosphate.

The quantification of glycolipids, either in total lipid extracts or fractions, may be estimated from the sugar amount determined using the orcinol colorimetric method [248]. A proportion of dry lipid samples is mixed with the orcinol solution (0.2% in 70% H_2SO_4) and heated to promote the hydrolysis of the glycolipids and the formation of sugar derivatives (with absorbance at 505 nm). D-glucose is commonly used as standard to obtain the calibration curve. The conversion factor 100/35 (ca. 2.8) is used to estimate the glycolipids amount in the samples [249].

3.3.2. Gas chromatography coupled to mass spectrometry (GC–MS) or flame ionization detector (GC-FID)

GC and GC–MS represent powerful tools for the quantitative and structural analysis of plant and algal lipids. As most lipids are not volatile, the analytes need to be derivatized to enable the transition of the molecules into the gas phase [250]. GC coupled with FID or MS are primarily in use for the separation and identification of FA, long chain base of SPs and phytosterols.

For FA analysis, FA residues (acyl groups) first undergo a de-esterification reaction to detach them from neutral and polar lipids, followed by transesterification to methanol, forming methyl esters. This reaction could be achieved on lipid extract or directly on the biomass [251]. Fatty acid methyl esters (FAMEs) with relatively low molecular weight are volatile and can then be analyzed using a GC system (GC–MS or GC-FID). This reaction is catalyzed by either a strong base or a strong acid, usually using NaOH or HCl or H_2SO_4, respectively [251]. FAMEs could be also converted into DMOX derivative and analyzed using GC–EI-MS to position the unsaturation on the carbon backbone [252]. For GC column selection, due to the high variety of fatty acid in plants and algae, columns of the highest polarity (e.g. CP-Sil 88™, BPX70™, or SP-2340™), are preferred [253].

Sterols are usually analyzed as either trimethylsilyl (TMS) ethers or as sterol acetates, which improves their volatility, peak shape, and response factors, compared to analysis of underivatized sterols [254,255]. In contrast to those used in fatty acid analysis, GC columns from low to medium polarity (e.g. DB-1™, or HP-5MS™) are preferred [111,122].

Derivatizations are usually reactions in which a significant amount of unreacted material remain in equilibrium. However, all FAs and STs have similar propensity to react with the derivatizing agents, so the lack of complete conversion of the compounds to the derivatized form does not present a problem. So, if a non-derivatized internal standard, absent from the sample (usually a FA with an odd carbon chain or cholestane), is added to the sample prior to the derivatization step, the overall fatty acid or sterol contents can then be calculated according to its relative peak area.

For SL long chain base analysis, a rapid three-step protocol involving the release of LCB from biological samples, their oxidation into aldehydes, and the subsequent separation on medium polarity column such as HP-5MS™ followed by identification/quantification of these aldehydes by GC–MS was developed by Cacas et al. [256].

Waxes are also analyzed by GC-FID and GC–MS on a HP-1™ column after a rapid chloroform extraction of plant tissues to extract soluble surface lipids [257]. Suberins and cutins are obtained after a thorough extraction of soluble lipids of plant tissues in increasing hydrophobicity solvents, the residue composed of suberin and cutin is further hydrolyzed by hot acidic methanol hydrolysis before being derivatized and analyzed by GC–MS on a HP-5MS™ column [44].

GC–MS allows untargeted analysis, due to the use of MS libraries and identification of molecular ion and MS fragmentation pattern and electron impact mass spectra, while GC-FID is usually used for targeted analysis. In GC-FID identification/annotation is only based on retention time and/or retention index. The separation and annotation of structural isomers can be particularly challenging and requires a good separation technique alongside using other annotation tools mentioned [252]. However, the linear range of the FID is better than the MS and allows quantification with higher accuracy when compounds are present in a wide dynamic range [258], as reported in olive oils, where FA 18:1 can represent more than 80% of total fatty acid and FA 16:1 represents less than 1% [259].

3.3.3. Lipidomic analysis

3.3.3.1. Direct infusion (DI) mass spectrometry (MS). Analysis of lipid extracts by direct infusion (DI) using ESI-MS instruments is limited by higher ion suppression effects compared to the LC-MS analysis, as well as the existence of isomeric lipids, impacting the sensitivity and selectivity of the analysis [260]. Whereas DI-MS is preferred in the analysis of purified lipid fractions, e.g., TG of olive oil [261], DI-MS can also be used in shotgun approaches for targeted lipid analysis. It is a very fast technique, compared with LC-MS, which is especially advantageous for routine analysis, e.g. as proposed for quality control of seed oils [262]. DI-MS analysis of lipid extracts is commonly used for targeted approaches, where the focus is the detection and quantification of a panel of specific lipid molecular species. For that, semi-targeted MS approaches with precursor ion scan (PIS) or neutral loss scan (NLS) are used for analysis of specific classes, e.g. in wild-type cotton (*Gossypium hirsutum*) [263]. The advantage of this approach is that it is targeted to a lipid class, but not to every lipid molecular species as it would be for multiple reaction monitoring (MRM) scan mode. This is possible because the lipid classes are not eluted on different chromatographic peak with different retention time. However, quantification can also be performed by MRM [260], as reported for the analysis of lipid changes after leaf wounding in *Arabidopsis thaliana* [264]. An additional advantage of DI over LC methods is the scan time which is not constrained by the elution time of a chromatographic peak, allowing much more multiple reaction monitoring (MRM) and therefore molecule detection and/or quantification than in LC-MS. A non-targeted approach could be also used for lipid discovery, but it would require high resolution mass spectrometry and accurate databases with plant or alga lipids. Due to the non-exhaustive presence of plants and alga lipids in databases, even if it improved a lot recently in database such as LIPIDMAPS or SwissLipids, the correct annotation is quite often difficult and requires an in-house database [265].

3.3.3.2. Liquid chromatography coupled to mass spectrometry. Liquid chromatography (LC)–mass spectrometry (MS) using non-targeted approaches are commonly used in plant [10,266,267] and alga (macroalgae [164,204,228,266,268,269] and microalgae [160,270–272]) lipidomic studies. LC-MS enables the separation of the lipid molecules before injection into the mass spectrometer, overcoming the ion suppression effects of DI. Reverse phase (RP)-LC-MS, normal phase (NP)-LC-MS, and hydrophilic interaction liquid chromatography (HILIC)-MS have been used for plant and algal lipid analysis, in different MS instruments. The columns used in LC-MS analysis vary in length, diameter, and particle size of the stationary phase as well as on the mobile phase's flow rate and composition (predominantly solvents compatible with ESI, such as methanol, acetonitrile) [273]. In NP-LC-MS [139] or HILIC-MS [10,141,160,164,204,228,267–272,274–277], the elution of the lipid molecules is based on their polar head moiety, separating the lipid molecules by lipid classes found in the lipidome of plants [10,139,267,274,276,277] and algae [141,160,164,204,228,268–270,272,275]. These columns are generally used for the analysis of samples with low amounts of neutral lipids (e.g., TGs) [62]. In RP-LC-MS, lipid molecules are separated based on the length, unsaturation degree, and position of the double bond of FA chains. Thus, RP-LC-MS enables the separation of lipid species and not separation of the different lipid classes [62]. The RP columns used in lipidomics of plants and algae

contain different modified sorbents (mainly C8 [278–282], C18 [218,283] and C30 [274,277] hydrocarbon chains). RP-LC-MS approaches have the advantage of allowing the separation of isomers and isobaric lipid species present in lipidome of plants and algae [62]. The lipid species esterified with longer and SFA chains have increased retention times (RT) (elute later) than lipid species bearing shorter and PUFA chains. Also, polar lipid species show shorter RT than neutral lipids [62]. RP-LC-MS is suitable for the analysis of polar and neutral lipids, but the chromatographic peaks of some lipid species from distinct lipid classes can overlap, making their accurate identification a difficult task [62]. Of note, a two-dimensional chromatographic setup coupling both RP and HILIC was already employed in the analysis of lipid extracts from rice samples (*Oryza sativa* L.) [276].

Development of supercritical fluid chromatography (SFC) is also an interesting approach for lipidomic analysis. SFC is considered a hybrid of GC and LC and has many advantages, such as high separation efficiency, low organic solvent consumption, and short analysis time. It is particularly well suited for the analysis of low-polarity compounds, such as lipids, because supercritical CO_2 is a non-polar solvent, often compared with hexane. Algae lipids could be separated and identified by SFC-ESI-MS with a NP column and the addition of ethanol as co-solvent for elution gradient [284].

LC is easy to combine with ESI, the predominant ionization technique in mass spectrometers used in LC-MS approaches for lipid analysis nowadays [11]. High resolution or triple quadrupole mass spectrometers are routinely used allowing respectively untargeted and targeted analysis. Untargeted LC-ESI-MS strategies are mostly done using data dependent acquisition (DDA) and/or data independent acquisition (DIA) modes (as recently reviewed by [11,62]. These acquisition methods generate a large amount of information that can be analyzed with the support of bioinformatic tools (e.g., MS-DIAL, MZmine, Lipostar). Identification is based on RT, exact mass, and MS/MS matching. Nevertheless, it is a good practice to validate the information provided by the software with a manual analysis of tandem MS spectra and/or, if available, with a lipid extract of known composition, because the lipid molecules present in plants and algae are poorly represented in lipid databases [62,66]. However, these techniques have the advantage of allowing the identification of hundreds of lipid molecular species from several classes, and thus to obtain a comprehensive view of the lipidome of plants and algae [62]. Nevertheless, quantification remains a challenge due to the lack of pure and well-characterized commercial standards for GL and BL and so most of the data reported in the literature are from relative quantification [62].

Targeted LC-MS approaches are used mainly on model organisms. Indeed, it is based on a list of MRM transition and therefore on a finished list of molecules to be analyzed. Therefore, the lipidome needs to be known before using this type of analysis [11,66]. Nowadays, LC-MS is mainly used for glycerolipid analysis but could be used also for fatty acid steryl esters, steryl glycosides and GIPC [113,123,285].

3.3.3.3. Quantification by mass spectrometry. Quantification by mass spectrometry is a challenge that should not be underestimated. Indeed, the molecule ionization efficiency is dependent of the matrix, the chain length, the number of unsaturation, the nature of the polar head, etc. [286]. Therefore, accurate quantification in lipidomics requires a method to control the variability of lipid extraction, ionization efficiency and systemic drift in the mass spectrometer. The addition of internal standards to the samples makes it possible to compensate for these sources of variability [287]. Ideally, internal standards should have the same chemical and physical properties as the lipids to be quantified, but distinguishable in mass spectra. Therefore, a stable-isotope labelled internal standard for each lipid species is the preferred option for accurate, but also costly, quantification [286]. However, lipidomic experiments measure several hundred of lipid molecules and stable-isotope labelled molecules are not available for each lipid molecule. In practice, it is common to use one or two internal standards for each lipid class, which are absent in the studied lipid extract, usually with odd or short chain [288].

For lipids, we can distinguish two cases: simple lipids; such as sterols and FA with their derivatives, and complex lipids; such as sphingolipids or glycerolipids. In the former, pure analytical standards are available to prepare calibration curves. In the last, hundreds of molecular species for each family can be detected for complex lipids in a biological sample with very few analytical standards commercially available. Under these circumstances, no calibration curves for each molecular species could be obtained, and because detection is sensitive to the nature of the molecule (especially with FAs), the absolute quantification will not be possible. To circumvent the discrepancy of ionization efficiency and the absence of available labelled standard for all lipid molecules, a quantified control (QC) sample can be used to normalize and correct the quantification. This QC corresponds to a known lipid extract mimicking the studied samples, quantified once by TLC plus GC-FID, and then systematically run with the samples to be analyzed by LC-MS/MS [66]. This method was established and validated for at least five kinds of organisms covering plants, microalgae and yeasts: *Arabidopsis thaliana, Nannochloropsis* sp.,*Phaeodactylum tricornutum, Aurantiochytrium limacinum* and *Saccharomyces cerevisiae*.

3.3.4. Mass spectrometry imaging (MSI)

Mass spectrometry imaging (MSI) is a technique used to map the spatial-temporal distribution of metabolites and proteins. It has been gradually applied in plant research in the past two decades, allowing researchers to investigate the distribution of biomolecules in all major plant organs and to track compositional changes in response to biotic and abiotic stresses (for recent reviews, see: [289–291]). MSI has been extensively used to study plant lipids, among them PLs, sulfolipids, SLs, and TGs [292,293]. Very few studies have used MSI to analyze algal lipids [154].

The three ionization techniques most used for MSI analysis are matrix-assisted laser desorption ionization (MALDI), desorption electrospray ionization (DESI) and secondary ion MS (SIMS). They differ in their spatial resolution, sensitivity, analysis speed and sample preparation procedure [290,294]. Most studies of plant lipids use MALDI-MS and DESI-MS [295]. Despite the high special resolution obtained in SIMS experiments (around 100 nm), the nanoscale SIMS (NanoSIMS) instruments ionize molecule by fragmenting them at masses below 200 Da and is therefore not suitable for lipid analysis. [234].

MALDI is a soft ionization technique performed either under vacuum or under ambient conditions, in which a chemical matrix coats the sample and promotes ionization of the metabolites through absorbing UV or IR laser energy [297,298]. It is currently the most widely used technique for studying lipid distribution and biochemistry in plant samples, with current state-of-the-art MALDI-MS instruments reaching a spatial resolution of a few micrometers and better sensitivity [299,300]. Sample preparation requires sectioning of the samples into thin slices that are then coated by the matrix. In plants, cryosectioning is commonly used to prepare tissue slices of leaves, flowers and fruits, whereas in microalgae dried cultures grown on a solid medium were previously used [154,294]. Matrix selection can greatly affect the detection of various lipid classes [301].

DESI is also a soft ionization method, performed under ambient conditions. It does not require a matrix but operates through the generation of charged microdroplets. The microdroplets are in direct contact with the sample surface, causing desorption of the metabolites [302]. The spatial resolution of DESI is lower than MALDI (usually 10–50 μm), however, it requires little sample preparation, making it suitable for surface analysis of lipids in soft plant tissues, such as petals and leaves, as well as direct analysis of algal cultures grown on solid medium [154,303]. Different solvents can be used to extract and ionize different lipid classes [304–306].

Recent advancements in MSI instrumentation and methodologies,

such as MALDI-2 and Tof-SIMS, incorporation of ion mobility and parallel MS and MS/MS imaging, hold promise to improve the detection and annotation of diverse lipid classes [296,307,308]. Applying them to plants and algae can greatly improve our understanding of the cellular lipid composition and the involvement of lipids in biological processes.

3.3.5. *Applications of Raman microscopy for lipidomics of microalgae*

Confocal Raman microscopy (CRM) is a contactless, non-invasive, and often non-destructive imaging method that combines the molecular specificity of Raman spectroscopy with the spatial resolution of confocal optical microscopy. CRM provides chemical images of the cells without specific staining or demanding preparatory procedures. The main advantage of CRM and related techniques consists in a simple and fast sample preparation, as well as multiplexing capability, i.e., identifying neutral lipids in the context of other storing bodies and intracellular structures (Fig. 13).

The first use of CRM for microalgae dates to forty years ago [309], however the routine usage in studies of algal lipids [310–312] has lagged due to problems caused by the inherent fluorescence of chlorophylls, and for a long time was limited only to carotenoids that provide sufficiently strong Raman signals to overcome this problem. Recent technological and methodological progress enabled the detection of chemical compounds exhibiting much weaker Raman scattering under the condition that within the cells they form densely packed or crystalline inclusions. CRM as well as macroscopic Raman spectroscopy are commonly used to detect and quantify neutral lipids (triacylglycerols) [313–319], often concurrently with starch [315,318,320], polyphosphates [321,322] or other biomolecules [323–325]. Since Raman signal is linear with concentration, CRM is suitable also for quantitative studies of lipids. The Raman estimates were often compared and validated by standard bulk quantification analyses, such as enzymatic analysis [320,321], GC–MS [315,317,318,326], LC-MS [313,314], gravimetry [327] or Nile Red staining [316] as a reference method for lipid quantification.

Raman techniques can also be used for a contact-less estimation of a mean unsaturation of algal lipids [310,314,317,327–330], and of other corresponding parameters, such as thawing temperature, mean number of C=C bonds and their ratio to CH_2 groups in FAs [328,330]. To estimate iodine value and other corresponding characteristics, the ratio of Raman bands of C=C stretching (1650–1660 cm^{-1}) and CH_2 bending (1440–1445 cm^{-1}) of microalgal lipids can be used [310,314,317,327–330] (Fig. 13). In vitro acquired spectra of 5–11 model fatty acids with different number of double bonds and chain lengths have been used to construct linear calibration curves for the saturation characteristics [310,314,317,327–330]; for thawing temperature estimation, a sigmoidal calibration curve was constructed [328,330]. Wu et al. compared thawing temperature of algal lipids obtained by Raman measurement and by calorimetry, and the two agreed within 1 °C [328].

Algal lipids were often studied in connection with carotenoids sequestered in the lipid droplets. The distributions of astaxanthin and lipids during *Haematococcus pluvialis* encystment were studied by Li et al. [331], and carotenoid concentration in lipid bodies has been quantified via the ratio of carotenoid and lipid bands [332]. Lipid bodies have also been imaged and quantified via advanced methods of Raman spectroscopy, e.g., using 2D and 3D coherent anti-Stokes Raman microscopy (CARS) [326,327], stimulated Raman spectroscopy (SRS) [333] and laser-trapping Raman spectroscopy (LTRS) [314,328,334]. Fatty acid composition of algal lipid bodies was also revealed by decomposing the Raman spectra of cellular lipids to spectra of the three or four most abundant fatty acids and compared with values obtained by GC–MS [317,329]. Apart from algal lipids, botryococcenes, liquid

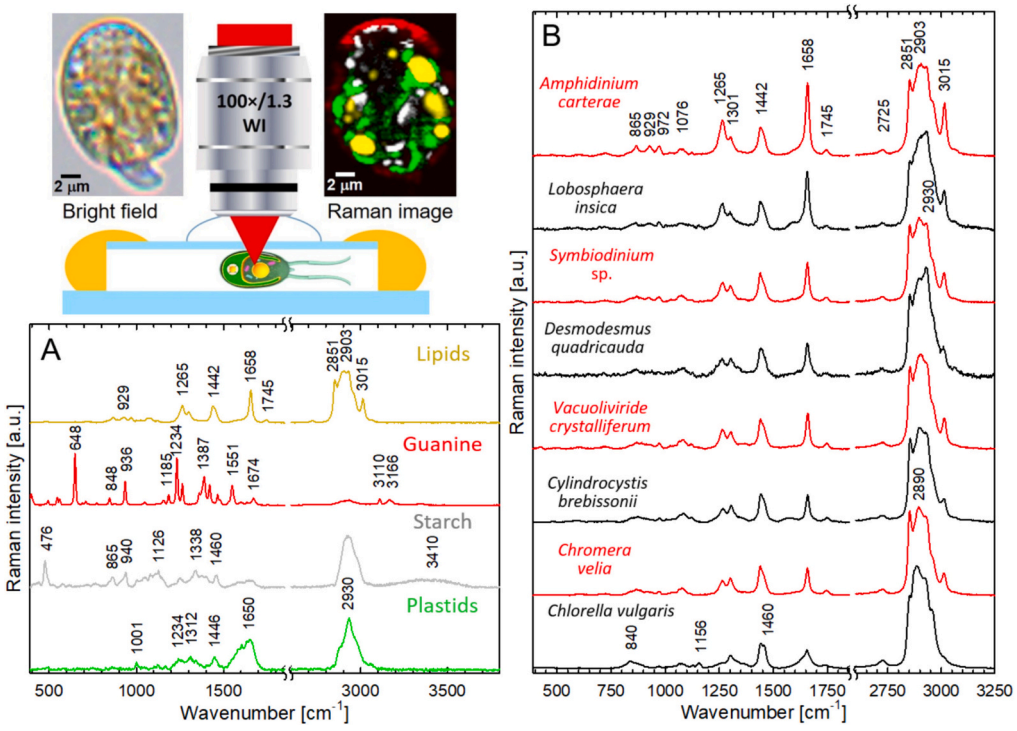


Fig. 13. Schematic illustrating the use of confocal Raman microscopy as a practical tool providing information on the composition of lipid bodies of microalgae in situ, in the context of other energy- and nutrient-storing biomolecules. Based on the characteristic Raman spectra of individual chemical components (panel A: yellow - neutral lipids, grey/white - floridean starch, red - crystalline guanine, green - plastids), Raman chemical image of a single cell *Amphidinium carterae* was constructed (the same colour code as in panel A). Spectral differences in the Raman spectra of lipid bodies of different microalgal species acquired in situ, i.e., at the single-cell level and without laborious extraction, reflect differences in their chemical composition, especially the different average degrees of saturation (panel B: see variable intensity ratio of the 1658 and 1442 cm^{-1} Raman bands). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hydrocarbons produced by *Botryococcus braunii*, were also studied by CRM [309,312].

3.3.6. Fourier-transform infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy is an excellent alternative method as an accurate method for lipid quantification in biomass, when screening for the best lipid producing species and optimizing their growth conditions. In lipid extract, infrared spectroscopy methods can be used for identification and for semi- or quantitative estimation of fatty acids, TGs, edible oils, etc., as well as for search of strains capable of accumulating or overproducing these particular metabolites [335–338].

FTIR spectroscopy is used as a label-free and fast analytical method providing objective and reliable information in a relatively non-invasive way. The main advantages of FTIR spectroscopy are as follows: (i) minimal sample pre-treatment and no chemical treatment, thus avoiding secondary reactions, (ii) significantly lower sample amounts that can be analyzed using ATR-FTIR (Attenuated Total Reflectance-FTIR), or FTIR-microspectroscopy using a miniature diamond anvil cell, (iii) presentation of all constituents of interest in a single spectrum, and (iv) capacity of high-throughput analysis and screening of a large number of samples [335,339,340]. FTIR spectrum of bio-samples provides information on a range of vibrationally active functional groups: O—H, N—H, C═O, =C–H, $–CH_2$, $–CH_3$, C–O–C and > P=O, in cells, isolated macromolecules, chemical constituents, biopolymers, nucleic acids, proteins, carbohydrates, lipids, etc. [341–344]. Chemical bonds within the functional groups of biochemical molecules have distinct vibrational properties, and thanks to that the relative amounts of these macromolecules can be well identified in spectrum. The principal components of the cell in the FTIR spectrum (Fig. 14) are identified by their absorption bands at: 1080 cm^{-1} for carbohydrates, 1250 cm^{-1} for nucleic acids, ~1660 and ~ 1550 cm^{-1} for proteins (the bands Amide *I* and Amide II stretching vibrations of C═O bond of amide and bending vibrations of the N—H bond, respectively), triplet bands in the region of 2800–3000 cm^{-1} (C—H stretching in CH_3 and CH_2) for the total lipids/fatty acids. The peak at 1744 cm^{-1} is assigned to C═O of esters/ester carbonyl and ~ 3014 cm^{-1} to olefinic HC═CH stretching mode, typical of unsaturated fatty acids [336,341,342,345].

It should be noted that the infrared spectrum of biomass, as a multicomponent system, represents a superposition of the cell components, and the snapshot (Fig. 14) reflects the distribution of macromolecular pools. The environmental stoichiometry of nutrients influences the relative abundance of organic pools in the cells of micro-, macroalgae, or phytoplankton cells that can be easily detected and analyzed by FTIR spectroscopy.

3.3.7. Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) is an emerging and powerful tool in lipidomic approaches. NMR does not require laborious or complex steps of sample preparation (e.g., fractionation, chromatographic separation, derivatization, or labelling). Both liquid and solid samples can be analyzed by NMR. This is a non-destructive quantitative spectroscopic technique, enabling further analysis of the same samples by other techniques, such as LC-MS or GC–MS. However, the major drawback of NMR is the high amount of sample necessary for each analysis [347].

In the latest years, proton (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) have been applied in the screening of algal lipids, namely for structural characterization and determination of the lipid content. ^{1}H NMR was used for the identification and quantification of free FAs and different glycerolipids species, including TGs, glycolipids and PLss from oleaginous microalgae, namely *Thalassiosira weissflogii*, *Cyclotella cryptica* and *Nannochloropsis salina* [348]. Both ^{1}H NMR and ^{13}C NMR were applied to evaluate the changes on the neutral and polar lipids content as well as in the unsaturated fatty acid profile of two microalgae, *Scenedesmus ecornis* and *Chlorella vulgaris* cultivated under different media [349]. Lipid extracts obtained from the red macroalga *Gracilaria longa* were also characterized by ^{1}H NMR and ^{13}C NMR. The ^{1}H NMR spectra provided information on the quantitative estimation of cholesterol and phosphatidylcholine/total lipid molar ratio, while ^{13}C NMR allowed identifying STs, chlorophylls, carotenoids, and glycolipids as well as to determine the position of the double bonds on the FAs [350]. ^{1}H, ^{13}C, and ^{31}P NMR have also been extensively used for the analysis of plant-derived oils, such as olive oil, to evaluate composition, quality and authentication [351,352]. For example, ^{31}P NMR was used for the identification, characterization, and quantification of the different PL classes while one- and two-dimensional ^{1}H NMR allowed the screening of the composition of fatty acids esterified in lipid classes (PL, TG) [353]. Finally, ^{31}P NMR could be used simply to quantify PL in lipid extract [354].

NMR could also be used for in vivo or biophysical analysis. For example, by ^{1}H pulse field gradient nuclear magnetic resonance, the size, the lipid content and the connections between lipid droplets have

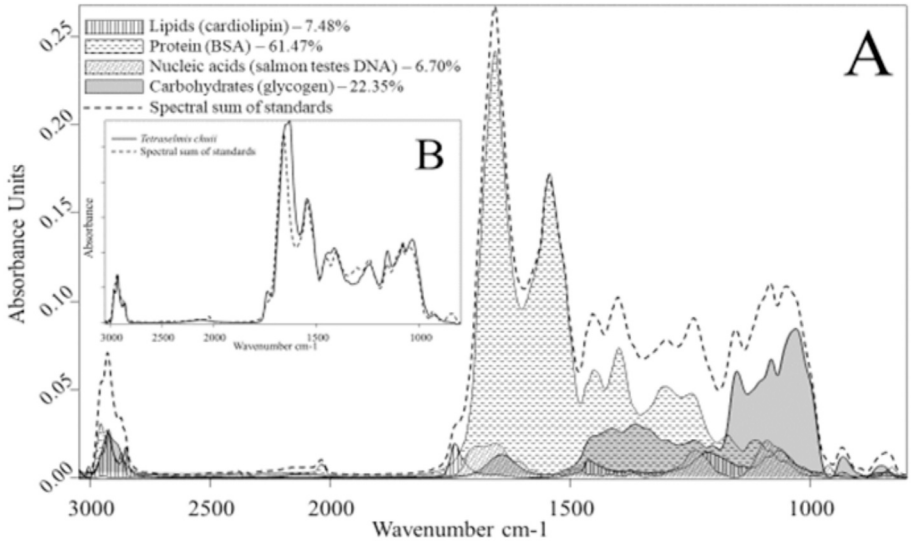


Fig. 14. A - Overlapping absorbances of the macromolecular components in the FTIR spectrum: carbohydrates, DNA, proteins, and lipids, in artificial mixture; B - FTIR spectrum of microalgae Tetraselmis chuii biomass and the spectral sum of standards (one of each class) in concentrations estimated by quantitative analysis (% dry weight), as in [346].

been measured in *Phaeodactylum tricornutum* cells [103]. By ^{31}P and ^{13}C NMR, the phase behavior of membrane composed of PL, GIPC and galactolipid was explored [39,355].

4. Plant and algal lipids: industrial applications

Why is it important to study lipid metabolism and profiling in plants and algae from a societal perspective? There are several reasons: health, nutrition, sustainable production of valuable compounds and green chemistry. From a nutritional point of view, several FAs are considered essential due to that they are poorly synthesized by human cells. The current main dietary source for VLC-PUFA is fish oil. Alternative and more sustainable sources of nutritional, healthy and bioactive lipids have to be found to fulfill the high societal demand for these compounds. Marine algae fresh microalgae, and other marine protists are likely candidates. Today, they are emerging as interesting alternatives for the production of a number of valuable compounds, including VLC-PUFA (human health), squalene (cosmetic industry), carotenoids and pigments and lecithins (phospholipids) (food industry). Plants and algae are the sustainable source of lipids for plant-based diets and replace animal compounds in a wide range of industries. Plants and algae are also promising vehicles for the development of non-petroleum chemistry. In this context, a chemistry based on renewable lipids from plants or algae could replace the non-sustainable use of fossil carbon, and indirectly contribute to the reduction of CO_2 emissions.

4.1. Plants and algae as a source of valuable lipids for food and feed

Plant and algal lipid applications in the food and feed industry are broad, from an edible foodstuff as its whole up to the production of a highly specialized molecule. As a raw material, edible plant lipids include mostly oils, liquid at room temperature, comprised mainly of unsaturated FA in a TG structure. The most abundant vegetable oils from a nutritional point of view worldwide are olive, sesame, palm, rapeseed (canola), soybean, sunflower, and corn, while oil from other origins (e.g., almond, safflower) although available, is less common [356]. Algal oil is only recently becoming available as an edible oil [357,358]. Additionally, common vegetable fats (solid at room temperature), with saturated FA as the main component, include shea, coconut, and cocoa, as well as vegetable oil margarines and vanapasti (vegetable ghee). Some plant lipids, e.g., jojoba and carnauba, are waxes, structured as an ester of a fatty acid and a fatty alcohol, and have various uses as food additives. e.g., for coatings [359]. Plant lipids also play a crucial role as raw material for other processes, including baking, frying, roasting and emulsifying [360,361]. As food ingredients, lipids significantly contribute to both food texture and flavor, providing characteristic mouthfeel, as well as precursors for aroma formation.

From a nutritional perspective, food lipids supply the human body with nutrients required for proper functioning, including essential FAs (ω-6 LA and ω-3 α-ALA). Algal eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the backbone for very long chain ω-3 PUFA synthesis in the human body. Lipids also allow the uptake of fat-soluble vitamins (A, D, E, K), phytosterols and carotenoids, compounds highlighted for their beneficial health effects [362]. From a health-related perspective, research has established the detrimental effects of saturated and *trans* FA consumption, and its negative effects on cardiovascular health [363]. This is as opposed to mono and PUFA, which have been positively correlated with cardiovascular and coronary proper functioning, as well as longevity [364].

Lipidomics research in food and feed enables developing improved foodstuff with favorable nutritional and functional properties, mainly through transgenic modifications [364], altering the composition of edible oils. Such applications can direct lipid metabolism towards accumulation of desirable FA, e.g., VLC ω-3 PUFAs, EPA, and DHA in commercial crops, e.g., canola and camelina, providing sustainable plant-based sources for these FA [364,365]. Due to consumer reproval of GMO, these approaches are mainly used for feed [366,367]. Another implication is profiling and designing high-oleic crops, increasing oleic acid content and improving their health contribution [368,369]. Profiling of commercial oils is of value in itself, providing information regarding nutritional lipids [370] and also enabling identifying underutilized sources of edible oils [369,371,372]. Another significant contribution of lipidomics is the identification and quantification of oxidized lipids in common oils and evaluating them as dietary sources of oxylipins, e.g., when consumed raw or heated [373]. Lipidomic research also facilitates the synthesis of designer lipids, as novel health-related lipids [374] and allows the effect characterization of processing, e.g., extraction [375], roasting [376], and storage [377,378] on oil quality and composition, and may serve as a tool for authentication and traceability in foodstuff or food ingredients, e.g., in oil [261,262] and wheat grains [379]. Also, in spite of algae lipidomics to address the added value as food has been less described, the lipidomic characterization of edible alga have been performed, e.g. in edible seaweed *Ulva rigida* (sea lettuce [204]), *Porphyra dioica* (well known as Nori in sushi) [110] and in microalgae as *Chlorella vulgaris* [58] to enhance their added value and as promising tool to identity and traceability [62].

Algae and plants produce lipids with superior nutritional qualities that are sought after to be incorporated into animal feed in agriculture and aquaculture. Brown algae, e.g.*Saccharina japonica*, *Undaria pinnatifida* and *Sargassum natans*, contain large amounts of LC-PUFAs, especially EPA and AA [92]. The inclusion of *U. pinnatifida* into animal feed was found to enhance the immune response in pigs [380], while feed supplementation with *S. japonica* resulted in the improved physicochemical qualities of goat meat [381]. *U. rigida* dietary supplementation has been shown to result in good growth performance in carp [382]. Microalgae and cyanobacteria belonging to different species: *Chlorococcum*, *Nitzschia, Nostoc, Spirogyra*, *Scenedesmus* spp. among others, are being used as feed supplements in livestock and poultry farming. Positive effects on animal health have been observed, involving immunity, fertility and juvenile survival [27]. As an example, the polar lipid extract of *Chlorococcum amblystomatis* with high amount of ω-3 C16, C18 PUFAs and EPA has demonstrated antioxidant activity and anti-inflammatory potential [160]. The dietary application of *Chlorella* and *Scenedesmus* spp. as feedstock in sericulture has resulted in the accelerated growth and development of silkworm caterpillars, as well as increased yield from cocoons [27]. A lipidomic characterization of the uptake of ω-3 PUFA to quantify egg lipids from hens whose diet had been supplemented with flaxseed oil or a DHA-rich marine algal supplement, demonstrated that the latter gave superior results [383]. Various other effects have also been observed, including enhanced egg size and increased production [27].

4.2. Applications in pharmaceutics

Plants, algae (macroalgae and microalgae), and cyanobacteria are a rich natural trove of bioactive lipids, including FAs, their derivatives, and complex lipids such as PL and glycolipids that may be beneficial for human health, and for medicinal uses [384–389]. Exploration of plant and algal lipidomes is critical for discovering novel and fostering the pharmaceutical potential of bioactive lipids. Lipidomics has been applied to investigate the pharmaceutical properties of medicinal plants and to identify the molecule responsible of the therapeutic effect as well as their mechanism of action [390–394]. Photosynthetic microalgae have gained increasing attention as an alternative renewable, clean, and sustainable source of health beneficial PUFA and VLC PUFA. VLC ω-3 and ω-6 PUFA, their oxygenated derivatives and glycerolipids esterified with them, are the most studied bioactive lipids with pharmaceutical potential for the treatment and alleviation of inflammation, a common component of human diseases [395,396]. The diversity of oxylipins (eicosanoids) and specialized lipid mediators produced from ω-3 and ω-6 C20 and C22 VLCPUFA and their role in regulation of the inflammatory response (Fig. 15) have been extensively covered elsewhere

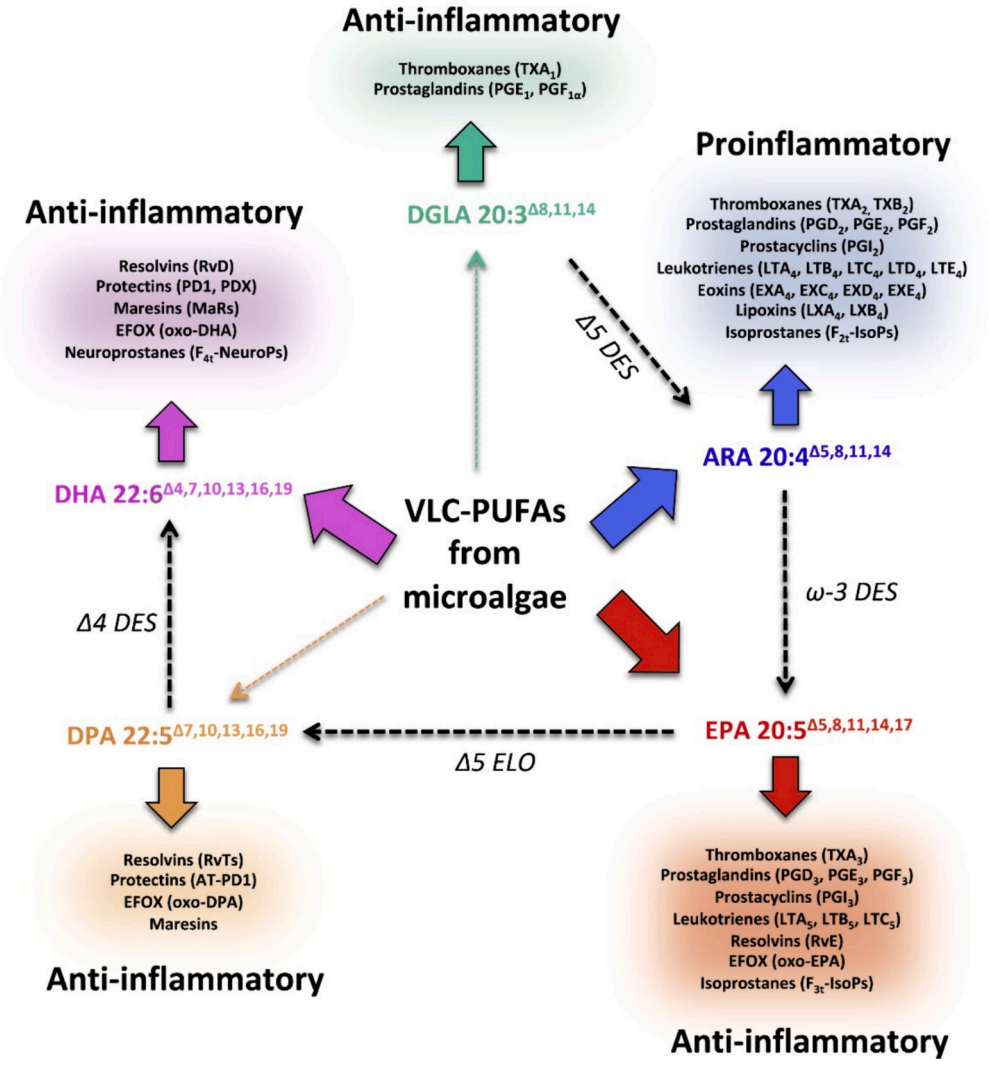


Fig. 15. Lipid mediators of inflammation derived from various omega-3 and omega-6 LC-PUFA. Adapted from Lupette & Benning [108].

[395,397–402].

Considering the importance of VLC PUFA for human health and of algae for addressing global challenges in food security and climate change, the lipidomes of VLCPUFA-producing microalgae and macroalgae have been investigated [15,106,401,403–406]. Microalgae and macroalgae may produce the oxygenated products of PUFA and VLC-PUFA enzymatically (oxylipins) [407] and non-enzymatically (isoprostanoids) [192,408], with high potential to modulate inflammatory responses. C18-PUFA and their oxygenated products were shown to exhibit anti-inflammatory activities [409–411]. High-ALA sage oil diet showed a decreased mucosal injury in a rodent model of Inflammatory Bowel Disease (IBD) compared to the corn and fish oil diets [409]. The anti-inflammatory activity of the green microalga *Chlamydomonas debaryana* in induced colitis [410,411] was associated with C18 PUFA-derived oxylipins, 13S- hydroxyoctadecatrienoic acid (HOTE) and 13S-hydroxyoctadecadienoic acid (HODE). 15S- hydroxyeicosapentaenoic acid (HEPE) from the EPA-producing microalga *Nannochloropsis gaditana*, decreased production of pro-inflammatory cytokines and expression of iNOS and COX-2 genes in macrophages [412]. Certain microalgae and macroalgae appeared to produce animal-like prostaglandins, derived from C20 LC-PUFA [407,413]. These findings highlight the therapeutic potential of plant and algal C18 PUFA and C20/C22 PUFA-derived oxylipins in inflammatory diseases such as IBD [414] and the importance of epilipidomics in uncovering this potential.

In addition, saturated and unsaturated FAs from plants and microalgae possess antibacterial activities against human bacterial pathogens [415,416] and as reviewed recently [417]. For example, non-esterified EPA liberated from the glycerolipids of the diatom *Phaeodactylum tricornutum* was effective against multidrug-resistant *Staphylococcus aureus*. Moreover, FAMEs extracted from the microalga *Scenedesmus intermedius* were active against a number of pathogenic bacteria and fungi [418]. Lipids from plants also exhibit antimicrobial properties. For example, heartwood lipid extract of *A. adianthifolia* [419] and steryl glycosides from roots of *B. portulacoides* [420] showed inhibiting capacity over *Escherichia coli* while SQDG isolated from neem (*Azadirachta indica*) showed antibacterial and antiviral activity [421].

4.3. Application in cosmetics

There is a constant market demand for the incorporation of natural ingredients in cosmetics formulations to enhance the quality, the efficacy and the environmental sustainability of the products [422]. However, for legislation simplicity, companies want to use products that are registered in the Inventory of Existing Cosmetic Ingredients in China (IECIC) that was revised in 2021. Plant extracts are highly detailed [423] and therefore there uses are restricted to those from particular plant varieties, whereas algae extract is present in the list without further detail (https://www.chinacosing.com/). Therefore, microalgae are

gaining increasing interest in cosmetics as they produce a variety of lipids (e.g., glycolipids, phytosterols,) with many potential uses such as emulsifying, antioxidant and anti-inflammatory agents [138,424–428].

Botanical oils are used in cosmetics as they have important biological properties for human skin [429]. Specifically, sunflower oil is a widely used inexpensive oil with high content of ω-6 LA [430]. In addition, flax or hemp oil, which are enriched with the ω-3 ALA, are used as anti-inflammatory and anti-comedogenic agents for skin [431]. Olive oil with high oleic acid content is considered as a deeply moisturizing factor in the three layers of skin while having anti-inflammatory and wound healing properties [430]. In addition, highly saturated coconut or shea butters are used to avoid the evaporation of water from the skin [429,432]. Jojoba wax was also recently reported to possess beneficial skin bioactivities, including anti-inflammatory and anti-herpes effects [433].

4.4. Applications in biofuels

Despite climate change, the gradual reduction in world reserves of fossil hydrocarbons added to the recent multiplication of health and geopolitical crises, the world demand for oil has never been as high as it is today [434]. Thus, it is therefore appropriate to find an alternative to fossil fuel energy, in particular through the third generation of biofuel derived from oleaginous microalgae. Indeed, the first two generations of biofuels come respectively from agricultural crops (production of biodiesel by transesterification of rapeseed oil, camelina oil, sunflower oil or palm oil) or plants rich in sugars for the first generation or from lignocellulosic waste for the second generation [97,434]. Competition between biofuels and food demand is inevitable with respect to food security issues. However, microalgae can achieve 1.83 kg of CO_2 biofixation in each kilogram of biomass, and its oil productivity is 10-fold higher than that of conventional biofuel crops [435]. Thereby, unlike the exploitation of fossil fuels which releases CO_2 trapped in geological time (Carboniferous, Jurassic and Cretaceous) and contributes to climate change, the exploitation of a marine algo-sourced biofuel does not compete with arable land, fresh water and does not emit more CO_2 than what was captured during photosynthesis.

To be usable for biofuel applications, FA esterified with the glycerol skeleton of TG must have two properties: i) a medium carbon chain (C8 to C14) in order to be compatible with the thermal properties of fuels, and ii) a low level of unsaturation in order to avoid oxidation problems [436,437]. However, these medium chain FA present two main biological problems: i) they are rarely found in the FA profiles of TG of oleaginous microalgae and ii) they are seldom tolerated by these microorganisms, leading to a cessation of the accumulation of biomass [438]. To counterbalance these biological locks, it is necessary to screen marine environments in order to identify in the phytoplankton biodiversity, more microalgae species that meet the research and industrial criteria for the generation of biofuels [97]. A second key consists in fully exploiting the genetic engineering tools (TALEN, CRISPR/Cas9, etc.) developed in recent years in many microalgae with the aim of reprogramming lipid metabolism [439]. For example, Radakovits and colleague showed that heterologous expression of two acyl-ACP thioesterases (TE) from camphor (*Cinnamomum camphora*) and California laurel (*Umbellularia californica*) in the pennate diatom *Phaeodactylum tricornutum* led to the incorporation of medium chain FAs (C12 and C14) in TG molecules but altered the accumulation of biomass [438]. Conversely, heterologous expression of *Uc*TE in *Chlamydomonas reinhardtii* does not alter the FA profile but overexpression of endogenous *Cr*TE promotes C14 accumulation [440]. A complementary study finally showed that the joint heterologous expression of a C10-C14 specific TE and ACP of cigar flower (*Cuphea lanceolata*) in *C. reinhardtii* slightly increased the quantity of C14 in TGs [441]. Finally, the recent discovery of a photoenzyme, fatty acid decarboxylase (FAP) in the microalga *Chlorella variabilis* NC64A and functionally characterized in *C. reinhardtii* opens up a wide range of perspectives [442,443]. Moulin and colleagues have identified 198 putative sequences of this photoenzyme, capable of converting FA into hydrocarbons by eliminating the carboxyl-end of FA, in data from the TARA Ocean scientific expedition as well as in sequenced microalgae genomes [443]. Other approaches that proved successful in upregulation of the TG levels include pharmacological inhibition of TOR (target of rapamycin) protein kinase by rapamycin, which resulted in TG accumulation in *C. reinhardtii* and *C. merolae* [444].

5. Challenges for tomorrow

5.1. Improve CO_2 capture and meet the need for food and feed

An increase in agricultural yield of 70% or more is required by 2050 to meet the growing demand of the world population [445,446]. Future gains face the challenges of shrinking farmland area, an increasingly unpredictable climate and the environmental imperative to use less fertilizer and agrochemicals, and will require new breeding strategies. To meet the strong societal demand for these various products, lipid production in plants and algae must be 'optimized'. Most sought-after phenotypic traits include oleaginous cells and tissues enriched in TGs (oil) or cells accumulating elevated levels of pigments (e.g. carotenoids, c-phycocyanin, astaxanthin). It is clear that the 'optimization' of these systems requires a complete understanding of i) their endogenous biosynthesis and regulation, and ii) their integration within the metabolism as a whole. This will then allow the rational development of the most appropriate engineering strategies to modify and adapt these organisms for commercial and societal purposes.

5.2. Uncoupling oil production and growth arrest

Plants and algae modulate and reprogram their metabolism to survive and proliferate under varying environmental conditions and nutrient availability. The metabolic plasticity of photosynthetic organisms involves the rearrangement and remodeling of the lipidome, that can be taken as an advantage when aiming to tune the production of lipids of interest for target industrial applications. However, the growth conditions for biomass production are often different than those for oil/lipid production. Lipidomics studies have provided novel insights into the dynamic responses of lipid classes and species and metabolic processes in terrestrial and aquatic plants, macroalgae, and microalgae [61,278,401,447–451].

Biosynthesis, accumulation, and restructuring of the lipid content of photosynthetic organisms are the main adaptive strategies triggered by exposure to stresses. In fact, one of the most popular strategies for inducing storage lipid synthesis in microalgal biotechnology is based on the exposure of microalgal cultures to different types of stress, mainly nutrient deprivation that has the major impact on blocking cell division [452]. Therefore, most of the strategies encountered today are done in two stages with a first phase for biomass production and a second stage for lipid production [453,454]. To tackle this challenge, modeling approach using a comprehensive and complementary lipidomic, genomic and transcriptomic knowledge could be used.

Increasingly available full genome sequences have made genome-scale metabolic modeling a more and more widely used approach [455]. This approach enables reconstruction of an explicit model of whole organism metabolism, based on sequence annotation data, and is already widely used in plants and algae [456,457]. A critical point in that respect is ensuring that already described metabolites in a given species are indeed present in the corresponding genome-scale metabolic network reconstruction. There are now databases making it possible to automatically assemble species-specific metabolomes although they are still in their infancy and biased towards well established genetic model species [458]. However, thanks to ongoing work on emerging model species, more datasets will certainly be integrated in the future. However, it is still challenging to connect data from genome-scale metabolic networks and from mass spectrometry databases. Even for well-studied

model species, the overlap is not more than 40% [459]. One of the reasons is the ambiguities still existing in molecule description in large-scale datasets. For example, the exact stereochemistry of a given molecule cannot be deduced directly from mass spectrometry data, and the lack of analytical standards make that many tentatively identified molecules could also correspond to other molecules with a similar mass-to-charge ratio. The other reason is that there is no biosynthetic model for a significant number of metabolites, including biologically important small molecules. This can be addressed by inferring ab initio new metabolite structures and new reactions based on genomic and metabolomic data [457]. Despite many other remaining challenges in integrating uncertainties [460], those approaches will certainly become more and more important in the future to identify what reactions are the bottlenecks for lipid production.

5.3. *Plants and algae lipidomics- sensors for climate changes*

Exposure to different stresses or adaptation to hostile environments can affect the lipid content and composition of algae and plants and causing an alteration of the typical lipid profile [461]. Thus, lipidomics has been used as a tool for assessing plant and alga adaptation to global climate changes and abiotically polluted environments.

In fact, the adaptation of plants and algae to unfavorable environments is indispensable for their growth and metabolic function. For example, these photosynthetic organisms can change or restructure the cell lipid composition as a measure of adaptation to abiotic stresses present in the environment. In this regard, global climate change arising from the rapid increase of greenhouse gases is provoking planet warming at unprecedented rates [462]. This global warming combined with local eutrophication caused by anthropogenic activities are leading drastic changes in marine ecosystems as species extinctions and ecosystem collapses [463]. Changes of temperature and nutrient uptake have been identified as some of the most relevant factors controlling plants, as well as algae and marine plant biological processes. In this regard, the global lipidomic map appears as a useful tool to understand the physiological response of (marine) plant and algal species under climate changes. Lipids may be used as biomarkers for the abiotic stress that these photosynthetic organisms must cope with these changes [7]. Besides, study of the plants and algae lipidome may facilitate an interesting approach to how its lipid biomarkers serve to assess their adaptive response to abiotically contaminated environments [464]. Decoding the adaptation of lipids to external stressors can be used to select more resilient species and also the best growth conditions to tune production of species with high content in lipids with specific properties for target applications.

To conclude, lipidomics has become an essential method for elucidating how terrestrial and marine plants and algae respond to different growth and environmental conditions. Insights into lipidome dynamics offer a deeper understanding of the plasticity of lipid metabolism in photosynthetic organisms, which is fundamental for their resilience and growth in a changing environment and biotechnological applications.

6. Concluding remarks

Plants and algae are the primary producers of lipids. These lipids exhibit a wide range of structural diversity. The lipidome of plants and algae contains several lipids shared with other biological kingdoms, such as triacylglycerides, sphingolipids, and phospholipids. On the other hand, some lipids are predominantly found within the Plantae kingdom, including glycolipids, betaine lipids, and specific sphingolipids (GIPC) and phytosterols. These lipids play vital roles in cellular membranes, organelles, and various biological processes, encompassing structural support, intercellular communication, lipid bilayer fusion, cytokinesis, vesicle trafficking, development, defense mechanisms, and responses to stress. While the functions of some lipids have been extensively studied, others have been overlooked in terms of their synthesis and roles. A substantial portion of our knowledge of lipid metabolism has been derived from plant studies. Nevertheless, an emerging field now focuses on the understanding of the lipidomics of algae.

The advancement of analytical techniques in the last decades has enabled the comprehensive characterization of these biomolecules, from their fundamental constituents, such as fatty acids, to the intricate structures found in glycerolipids, sterols and sphingolipids, as well as the corresponding epilipids generated by chemical modifications mediated by enzymatic or non-enzymatic mechanisms, primarily driven by processes like oxidation and nitration, with formation a variety of lipid oxidation (e.g. oxylipins) and nitrate lipids.

Various analytical methods have been employed for the identification and quantification of different lipid categories present in plants and algae. Mass spectrometry-based approaches have been among the most commonly used techniques. However, non-contact, non-invasive, or non-destructive methods, including Raman microscopy and NMR have also found application in the study of lipids.

The characterization of plant and algal lipidome is highlighting the traditional expertise on lipids derived from plants and algae, underlining their significant potential for diverse industrial applications. The lipidomes of these organisms stand out for their health-related nutritional value, primarily due to their high content of essential omega-3 and omega-6 VLC PUFAs, phytosterols, and fat-soluble vitamins. Lipids isolated from plants and algae have been associated with various bioactivities, including antioxidant, anti-inflammatory, and antimicrobial properties, underscoring their pharmaceutical potential. Furthermore, botanical and algal oils find applications in the cosmetic industry owing to their advantageous skin-related properties, such as anti-inflammatory and wound-healing attributes. The high productivity of plants and algae, along with their ability to sequester CO_2, has been proposed as an alternative to fossil fuels. However, the competition between biofuels and food resources has led to the exploration of new alternatives. Marine microalgae have emerged as a promising alternative to fossil fuels since they do not compete for arable land and freshwater resources.Nevertheless, there are several challenges that need to be addressed in this regard. These challenges include optimizing lipid production in plants and algae, which requires a more profound understanding of lipid metabolism within these organisms and their endogenous regulatory mechanisms. Additionally, it is essential to explore the metabolic adaptability of plants and algae under stress conditions and how lipids can serve as ecological biomarkers to enhance our understanding of planetary processes.

CRediT authorship contribution statement

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References

[1] Petroutsos D, Amiar S, Abida H, Dolch L-J, Bastien O, Rébeillé F, et al. Evolution of galactoglycerolipid biosynthetic pathways–from cyanobacteria to primary plastids and from primary to secondary plastids. Prog Lipid Res 2014;54:68–85. https://doi.org/10.1016/j.plipres.2014.02.001.

[2] Couto D, Conde TA, Melo T, Neves B, Costa M, Silva J, et al. The chemodiversity of polar lipidomes of microalgae from different taxa. Algal Res 2023;70:103006. https://doi.org/10.1016/j.algal.2023.103006.

[3] Ischebeck T, Krawczyk HE, Mullen RT, Dyer JM, Chapman KD. Lipid droplets in plants and algae: distribution, formation, turnover and function. Semin Cell Dev Biol 2020;108:82–93. https://doi.org/10.1016/j.semcdb.2020.02.014.

[4] Nakamura Y. Phosphate starvation and membrane lipid remodeling in seed plants. Prog Lipid Res 2013;52:43–50. https://doi.org/10.1016/j.plipres.2012.07.002.

[5] Kalisch B, Dörmann P, Hölzl G. DGDG and glycolipids in plants and algae. In: Nakamura Y, Li-Beisson Y, editors. Lipids in plant and algae development. Cham: Springer International Publishing; 2016. p. 51–83. https://doi.org/10.1007/978-3-319-25979-6_3.

[6] Van Mooy BAS, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, Koblízek M, et al. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. Nature 2009;458:69–72. https://doi.org/10.1038/nature07659.

[7] Holm HC, Fredricks HF, Bent SM, Lowenstein DP, Ossolinski JE, Becker KW, et al. Global Ocean lipidomes show a universal relationship between temperature and lipid unsaturation. Science 2022;376:1487–91. https://doi.org/10.1126/science.abn7455.

[8] Becker KW, Collins JR, Durham BP, Groussman RD, White AE, Fredricks HF, et al. Daily changes in phytoplankton lipidomes reveal mechanisms of energy storage in the open ocean. Nat Commun 2018;9:5179. https://doi.org/10.1038/s41467-018-07346-z.

[9] Li-Beisson Y, Thelen JJ, Fedosejevs E, Harwood JL. The lipid biochemistry of eukaryotic algae. Prog Lipid Res 2019;74:31–68. https://doi.org/10.1016/j.plipres.2019.01.003.

[10] Alves E, Rey F, Melo T, Barros MP, Domingues P, Domingues R. Bioprospecting bioactive polar Lipids from olive (*Olea europaea* cv. Galega vulgar) fruit seeds: LC-HR-MS/MS fingerprinting and sub-geographic comparison. Foods 2022;11:951. https://doi.org/10.3390/foods11070951.

[11] Kehelpannala C, Rupasinghe T, Hennessy T, Bradley D, Ebert B, Roessner U. The state of the art in plant lipidomics. Mol Omics 2021;17:894–910. https://doi.org/10.1039/D1MO00196E.

[12] Joubès J, Raffaele S, Bourdenx B, Garcia C, Laroche-Traineau J, Moreau P, et al. The VLCFA elongase gene family in Arabidopsis thaliana: phylogenetic analysis, 3D modelling and expression profiling. Plant Mol Biol 2008;67:547–66. https://doi.org/10.1007/s11103-008-9339-z.

[13] Lu Q, Li H, Xiao Y, Liu H. A state-of-the-art review on the synthetic mechanisms, production technologies, and practical application of polyunsaturated fatty acids from microalgae. Algal Res 2021;55:102281. https://doi.org/10.1016/j.algal.2021.102281.

[14] Abida H, Dolch L-J, Meï C, Villanova V, Conte M, Block MA, et al. Membrane glycerolipid remodeling triggered by nitrogen and phosphorus starvation in *Phaeodactylum tricornutum*. Plant Physiol 2015;167:118–36. https://doi.org/10.1104/pp.114.252395.

[15] Degraeve-Guilbault C, Bréhélin C, Haslam R, Sayanova O, Marie-Luce G, Jouhet J, et al. Glycerolipid characterization and nutrient deprivation-associated changes in the green Picoalga Ostreococcus tauri1. Plant Physiol 2017;173:2060–80. https://doi.org/10.1104/pp.16.01467.

[16] Dolganyuk V, Andreeva A, Budenkova E, Sukhikh S, Babich O, Ivanova S, et al. Study of morphological features and determination of the fatty acid composition of the microalgae lipid complex. Biomolecules 2020;10:1571. https://doi.org/10.3390/biom10111571.

[17] Ummalyma SB, Sindhu R, Binod P, Pandey A, Gnansounou E. Production and applications of polyunsaturated fatty acids. High value fermentation products. John Wiley & Sons, Ltd; 2019. p. 109–25. https://doi.org/10.1002/9781119555384.ch6.

[18] Lim G-H, Singhal R, Kachroo A, Kachroo P. Fatty acid– and lipid-mediated signaling in plant defense. Annu Rev Phytopathol 2017;55:505–36. https://doi.org/10.1146/annurev-phyto-080516-035406.

[19] Rawsthorne S. Carbon flux and fatty acid synthesis in plants. Prog Lipid Res 2002;41:182–96. https://doi.org/10.1016/s0163-7827(01)00023-6.

[20] Smith S, Tsai S-C. The type I fatty acid and polyketide synthases: a tale of two megasynthases. Nat Prod Rep 2007;24:1041–72. https://doi.org/10.1039/B603600G.

[21] Batsale M, Bahammou D, Fouillen L, Mongrand S, Joubès J, Domergue F. Biosynthesis and functions of very-long-chain fatty acids in the responses of plants to abiotic and biotic stresses. Cells 2021;10:1284. https://doi.org/10.3390/cells10061284.

[22] Roleda MY, Slocombe SP, Leakey RJG, Day JG, Bell EM, Stanley MS. Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. Bioresour Technol 2013;129:439–49. https://doi.org/10.1016/j.biortech.2012.11.043.

[23] Gao J, Ajjawi I, Manoli A, Sawin A, Xu C, Froehlich JE, et al. FATTY ACID DESATURASE4 of Arabidopsis encodes a protein distinct from characterized fatty acid desaturases. Plant J 2009;60:832–9. https://doi.org/10.1111/j.1365-313X.2009.04001.x.

[24] Dolch L-J, Rak C, Perin G, Tourcier G, Broughton R, Leterrier M, et al. A palmitic acid elongase affects eicosapentaenoic acid and plastidial monogalactosyldiacylglycerol levels in nannochloropsis. Plant Physiol 2017;173:742–59. https://doi.org/10.1104/pp.16.01420.

[25] Ohlrogge J, Thrower N, Mhaske V, Stymne S, Baxter M, Yang W, et al. PlantFAdb: a resource for exploring hundreds of plant fatty acid structures synthesized by thousands of plants and their phylogenetic relationships. Plant J 2018;96:1299–308. https://doi.org/10.1111/tpj.14102.

[26] Conde TA, Neves BF, Couto D, Melo T, Neves B, Costa M, et al. Microalgae as sustainable bio-factories of healthy Lipids: evaluating fatty acid content and antioxidant activity. Mar Drugs 2021;19:1–20. https://doi.org/10.3390/md19070357.

[27] Babich O, Sukhikh S, Larina V, Kalashnikova O, Kashirskikh E, Prosekov A, et al. Algae: study of edible and biologically active fractions, their properties and applications. Plants 2022;11:780. https://doi.org/10.3390/plants11060780.

[28] Praiboon J, Palakas S, Noiraksa T, Miyashita K. Seasonal variation in nutritional composition and anti-proliferative activity of brown seaweed, *Sargassum oligocystum*. J Appl Phycol 2018;30:101–11. https://doi.org/10.1007/s10811-017-1248-6.

[29] Ruffing AM, Jones HDT. Physiological effects of free fatty acid production in genetically engineered *Synechococcus elongatus* PCC 7942. Biotechnol Bioeng 2012;109:2190–9. https://doi.org/10.1002/bit.24509.

[30] Balduyck L, Dejonghe C, Goos P, Jooken E, Muylaert K, Foubert I. Inhibition of lipolytic reactions during wet storage of T-Isochrysis lutea biomass by heat

treatment. Algal Res 2019;38:101388. https://doi.org/10.1016/j.algal.2018.101388.
[31] Christie WW. Lipid analysis: Isolation, separation, identification, and structural analysis of lipids. Bridgwater, England: Oily Press; 2003.
[32] Cahoon EB, Li-Beisson Y. Plant unusual fatty acids: learning from the less common. Curr Opin Plant Biol 2020;55:66–73. https://doi.org/10.1016/j.pbi.2020.03.007.
[33] Scott S, Cahoon EB, Busta L. Variation on a theme: the structures and biosynthesis of specialized fatty acid natural products in plants. Plant J 2022;111:954–65. https://doi.org/10.1111/tpj.15878.
[34] Wattelet-Boyer V, Brocard L, Jonsson K, Esnay N, Joubès J, Domergue F, et al. Enrichment of hydroxylated C24- and C26-acyl-chain sphingolipids mediates PIN2 apical sorting at trans-Golgi network subdomains. Nat Commun 2016;7:12788. https://doi.org/10.1038/ncomms12788.
[35] Nagano M, Takahara K, Fujimoto M, Tsutsumi N, Uchimiya H, Kawai-Yamada M. Arabidopsis sphingolipid fatty acid 2-hydroxylases (AtFAH1 and AtFAH2) are functionally differentiated in fatty acid 2-hydroxylation and stress responses1 [OA]. Plant Physiol 2012;159:1138–48. https://doi.org/10.1104/pp.112.199547.
[36] Ukawa T, Banno F, Ishikawa T, Kasahara K, Nishina Y, Inoue R, et al. Sphingolipids with 2-hydroxy fatty acids aid in plasma membrane nanodomain organization and oxidative burst. Plant Physiol 2022;189:839–57. https://doi.org/10.1093/plphys/kiac134.
[37] Mamode Cassim A, Grison M, Ito Y, Simon-Plas F, Mongrand S, Boutté Y. Sphingolipids in plants: a guidebook on their function in membrane architecture, cellular processes, and environmental or developmental responses. FEBS Lett 2020;594:3719–38. https://doi.org/10.1002/1873-3468.13987.
[38] Nagano M, Ishikawa T, Fujiwara M, Fukao Y, Kawano Y, Kawai-Yamada M, et al. Plasma membrane microdomains are essential for Rac1-RbohB/H-mediated immunity in rice. Plant Cell 2016;28:1966–83. https://doi.org/10.1105/tpc.16.00201.
[39] Mamode Cassim A, Navon Y, Gao Y, Decossas M, Fouillen L, Grélard A, et al. Biophysical analysis of the plant-specific GIPC sphingolipids reveals multiple modes of membrane regulation. J Biol Chem 2021;296:100602. https://doi.org/10.1016/j.jbc.2021.100602.
[40] Marquês JT, Marinho HS, de Almeida RFM. Sphingolipid hydroxylation in mammals, yeast and plants – an integrated view. Prog Lipid Res 2018;71:18–42. https://doi.org/10.1016/j.plipres.2018.05.001.
[41] Chauke NP, Mukaya HE, Nkazi DB. Chemical modifications of castor oil: A review. Sci Prog 2019;102:199–217. https://doi.org/10.1177/0036850419859118.
[42] Broun P, Shanklin J, Whittle E, Somerville C. Catalytic plasticity of fatty acid modification enzymes underlying chemical diversity of plant Lipids. Science 1998;282:1315–7. https://doi.org/10.1126/science.282.5392.1315.
[43] Pollard M, Beisson F, Li Y, Ohlrogge JB. Building lipid barriers: biosynthesis of cutin and suberin. Trends Plant Sci 2008;13:236–46. https://doi.org/10.1016/j.tplants.2008.03.003.
[44] Delude C, Fouillen L, Bhar P, Cardinal M-J, Pascal S, Santos P, et al. Primary fatty alcohols are major components of suberized root tissues of Arabidopsis in the form of alkyl Hydroxycinnamates. Plant Physiol 2016;171:1934–50. https://doi.org/10.1104/pp.16.00834.
[45] Wellesen K, Durst F, Pinot F, Benveniste I, Nettesheim K, Wisman E, et al. Functional analysis of the LACERATA gene of Arabidopsis provides evidence for different roles of fatty acid ω-hydroxylation in development. Proc Natl Acad Sci 2001;98:9694–9. https://doi.org/10.1073/pnas.171285998.
[46] Höfer R, Briesen I, Beck M, Pinot F, Schreiber L, Franke R. The Arabidopsis cytochrome P450 CYP86A1 encodes a fatty acid ω-hydroxylase involved in suberin monomer biosynthesis. J Exp Bot 2008;59:2347–60. https://doi.org/10.1093/jxb/ern101.
[47] Compagnon V, Diehl P, Benveniste I, Meyer D, Schaller H, Schreiber L, et al. CYP86B1 Is required for very long chain ω-hydroxyacid and α,ω-dicarboxylic acid synthesis in root and seed suberin polyester. Plant Physiol 2009;150:1831–43. https://doi.org/10.1104/pp.109.141408.
[48] Li-Beisson Y, Pollard M, Sauveplane V, Pinot F, Ohlrogge J, Beisson F. Nanoridges that characterize the surface morphology of flowers require the synthesis of cutin polyester. Proc Natl Acad Sci 2009;106:22008–13. https://doi.org/10.1073/pnas.0909090106.
[49] Yore MM, Syed I, Moraes-Vieira PM, Zhang T, Herman MA, Homan EA, et al. Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. Cell 2014;159:318–32. https://doi.org/10.1016/j.cell.2014.09.035.
[50] Brejchova K, Balas L, Paluchova V, Brezinova M, Durand T, Kuda O. Understanding FAHFAs: from structure to metabolic regulation. Prog Lipid Res 2020;79:101053. https://doi.org/10.1016/j.plipres.2020.101053.
[51] Liberati A, Biluš M, Brkić AL, Barić I, Bakula M, Hozic A, et al. Analysis of fatty acid esters of hydroxyl fatty acid in selected plant food. Plant Foods Hum Nutr 2019;74:1–6. https://doi.org/10.1007/s11130-019-00728-8.
[52] Balas L, Bertrand-Michel J, Viars F, Faugere J, Lefort C, Caspar-Bauguil S, et al. Regiocontrolled syntheses of FAHFAs and LC-MS/MS differentiation of regioisomers. Org Biomol Chem 2016;14:9012–20. https://doi.org/10.1039/C6OB01597B.
[53] Chen Y, Biresaw G, Cermak SC, Isbell TA, Ngo HL, Chen L, et al. Fatty acid estolides: a review. J Am Oil Chem Soc 2020;97:231–41. https://doi.org/10.1002/aocs.12323.
[54] Bernard A, Joubès J. Arabidopsis cuticular waxes: advances in synthesis, export and regulation. Prog Lipid Res 2013;52:110–29. https://doi.org/10.1016/j.plipres.2012.10.002.
[55] Liebisch G, Fahy E, Aoki J, Dennis EA, Durand T, Ejsing CS, et al. Update on LIPID MAPS classification, nomenclature, and shorthand notation for MS-derived lipid structures. J Lipid Res 2020;61:1539–55. https://doi.org/10.1194/jlr.S120001025.
[56] Kobayashi K. Role of membrane glycerolipids in photosynthesis, thylakoid biogenesis and chloroplast development. J Plant Res 2016;129:565–80. https://doi.org/10.1007/s10265-016-0827-y.
[57] Hölzl G, Dörmann P. Chloroplast Lipids and their biosynthesis. Annu Rev Plant Biol 2019;70:51–81. https://doi.org/10.1146/annurev-arplant-050718-100202.
[58] Couto D, Melo T, Conde TA, Costa M, Silva J, Domingues MRM, et al. Chemoplasticity of the polar lipid profile of the microalgae *Chlorella vulgaris* grown under heterotrophic and autotrophic conditions. Algal Res 2021;53:1–16. https://doi.org/10.1016/j.algal.2020.102128.
[59] Figueiredo ARP, da Costa E, Silva J, Domingues MR, Domingues P. The effects of different extraction methods of lipids from Nannochloropsis oceanica on the contents of omega-3 fatty acids. Algal Res 2019;41:1–10. https://doi.org/10.1016/j.algal.2019.101556.
[60] Ahmmed MK, Ahmmed F, Tian H Sabrina, Carne A, Bekhit AE-D. Marine omega-3 (n-3) phospholipids: A comprehensive review of their properties, sources, bioavailability, and relation to brain health. Compr Rev Food Sci Food Saf 2020;19:64–123. https://doi.org/10.1111/1541-4337.12510.
[61] Tietel Z, Wikoff WR, Kind T, Ma Y, Fiehn O. Hyperosmotic stress in Chlamydomonas induces metabolomic changes in biosynthesis of complex lipids. Eur J Phycol 2020;55:11–29. https://doi.org/10.1080/09670262.2019.1637547.
[62] Rey F, Melo T, Lopes D, Couto D, Marques F, Domingues MR. Applications of lipidomics in marine organisms: progress, challenges and future perspectives. Mol Omics 2022;18:357–86. https://doi.org/10.1039/D2MO00012A.
[63] Gasulla F, García-Plazaola JI, López-Pozo M, Fernández-Marín B. Evolution, biosynthesis and protective roles of oligogalactolipids: key molecules for terrestrial photosynthesis? Environ Exp Bot 2019;164:135–48. https://doi.org/10.1016/j.envexpbot.2019.05.003.
[64] Okazaki Y, Otsuki H, Narisawa T, Kobayashi M, Sawai S, Kamide Y, et al. A new class of plant lipid is essential for protection against phosphorus depletion. Nat Commun 2013;4:1510. https://doi.org/10.1038/ncomms2512.
[65] Kim Y, Terng EL, Riekhof WR, Cahoon EB, Cerutti H. Endoplasmic reticulum acyltransferase with prokaryotic substrate preference contributes to triacylglycerol assembly in Chlamydomonas. Proc Natl Acad Sci 2018;115:1652–7. https://doi.org/10.1073/pnas.1715922115.
[66] Jouhet J, Lupette J, Clerc O, Magneschi L, Bedhomme M, Collin S, et al. LC-MS/MS versus TLC plus GC methods: consistency of glycerolipid and fatty acid profiles in microalgae and higher plant cells and effect of a nitrogen starvation. PLoS One 2017;12:e0182423. https://doi.org/10.1371/journal.pone.0182423.
[67] Block MA, Jouhet J. Lipid trafficking at endoplasmic reticulum-chloroplast membrane contact sites. Curr Opin Cell Biol 2015;35:21–9. https://doi.org/10.1016/j.ceb.2015.03.004.
[68] Bastien O, Botella C, Chevalier F, Block MA, Jouhet J, Breton C, et al. New insights on thylakoid biogenesis in plant cells. Int Rev Cell Mol Biol 2016;323:1–30. https://doi.org/10.1016/bs.ircmb.2015.12.001.
[69] Higashi Y, Okazaki Y, Myouga F, Shinozaki K, Saito K. Landscape of the lipidome and transcriptome under heat stress in *Arabidopsis thaliana*. Sci Rep 2015;5:10533. https://doi.org/10.1038/srep10533.
[70] Giossi CE, Cruz S, Rey F, Marques R, Melo T, Domingues M do R, et al. Light induced changes in pigment and lipid profiles of Bryopsidales algae. Front Mar Sci 2021:8.
[71] Moellering ER, Muthan B, Benning C. Freezing tolerance in plants requires lipid remodeling at the outer chloroplast membrane. Science 2010;330:226–8. https://doi.org/10.1126/science.1191803.
[72] Chen J, Burke JJ, Xin Z, Xu C, Velten J. Characterization of the Arabidopsis thermosensitive mutant atts02 reveals an important role for galactolipids in thermotolerance. Plant Cell Environ 2006;29:1437–48. https://doi.org/10.1111/j.1365-3040.2006.01527.x.
[73] Murakami H, Nobusawa T, Hori K, Shimojima M, Ohta H. Betaine lipid is crucial for adapting to low temperature and phosphate deficiency in nannochloropsis. Plant Physiol 2018;177:181–93. https://doi.org/10.1104/pp.17.01573.
[74] Sato N. Lipids in Cryptomonas CR-1. II. Biosynthesis of betaine lipids and galactolipids. Plant Cell Physiol 1991;32:845–51. https://doi.org/10.1093/oxfordjournals.pcp.a078152.
[75] Janero DR, Barrnett R. Isolation and characterization of an ether-linked homoserine lipid from the thylakoid membrane of *Chlamydomonas reinhardtii* 137+. J Lipid Res 1982;23:307–16. https://doi.org/10.1016/S0022-2275(20)38161-X.
[76] Zhong Y, Li Y, Xu J, Cao J, Zhou C, Yan X. Isolation of chloroplasts from marine microalga *Isochrysis galbana* Parke for their lipid composition analysis. J Ocean Univ China 2022;21:225–35. https://doi.org/10.1007/s11802-022-4834-4.
[77] Dorne AJ, Joyard J, Block MA, Douce R. Localization of phosphatidylcholine in outer envelope membrane of spinach chloroplasts. J Cell Biol 1985;100:1690–7. https://doi.org/10.1083/jcb.100.5.1690.
[78] Cañavate JP, Armada I, Ríos JL, Hachero-Cruzado I. Exploring occurrence and molecular diversity of betaine lipids across taxonomy of marine microalgae. Phytochemistry 2016;124:68–78. https://doi.org/10.1016/j.phytochem.2016.02.007.

[79] Lopes D, Melo T, Rey F, Costa E, ASP Moreira, Abreu MH, et al. Insights of species-specific polar lipidome signatures of seaweeds fostering their valorization in the blue bioeconomy. 2021.
[80] Coulon D, Faure L, Salmon M, Wattelet V, Bessoule J-J. *N*-Acylethanolamines and related compounds: aspects of metabolism and functions. Plant Sci 2012;184:129–40. https://doi.org/10.1016/j.plantsci.2011.12.015.
[81] Song Y, Zoong Lwe ZS, Wickramasinghe PADBV, Welti R. Head-group acylation of chloroplast membrane lipids. Molecules 2021;26:1273. https://doi.org/10.3390/molecules26051273.
[82] Riekhof WR, Ruckle ME, Lydic TA, Sears BB, Benning C. The Sulfolipids 2′-O-acyl-Sulfoquinovosyldiacylglycerol and Sulfoquinovosyldiacylglycerol are absent from a *Chlamydomonas reinhardtii* mutant deleted in SQD1. Plant Physiol 2003;133:864–74. https://doi.org/10.1104/pp.103.029249.
[83] Coulon D, Faure L, Salmon M, Wattelet V, Bessoule J-J. Occurrence, biosynthesis and functions of *N*-acylphosphatidylethanolamines (NAPE): not just precursors of *N*-acylethanolamines (NAE). Biochimie 2012;94:75–85. https://doi.org/10.1016/j.biochi.2011.04.023.
[84] Kilaru A, Tamura P, Isaac G, Welti R, Venables BJ, Seier E, et al. Lipidomic analysis of N-acylphosphatidylethanolamine molecular species in Arabidopsis suggests feedback regulation by N-acylethanolamines. Planta 2012;236:809–24. https://doi.org/10.1007/s00425-012-1669-z.
[85] De Luca L, Ferracane R, Vitaglione P. Food database of *N*-acyl-phosphatidylethanolamines, *N*-acylethanolamines and endocannabinoids and daily intake from a Western, a Mediterranean and a vegetarian diet. Food Chem 2019;300:125218. https://doi.org/10.1016/j.foodchem.2019.125218.
[86] Bianco M, Calvano CD, Ventura G, Bianco G, Losito I, Cataldi TRI. Regiochemical assignment of N-Acylphosphatidylethanolamines (NAPE) by liquid chromatography/electrospray ionization with multistage mass spectrometry and its application to extracts of Lupin seeds. J Am Soc Mass Spectrom 2020;31:1994–2005. https://doi.org/10.1021/jasms.0c00267.
[87] Rawyler AJ, Braendle RA. N-Acylphosphatidylethanolamine accumulation in potato cells upon energy shortage caused by anoxia or respiratory inhibitors. Plant Physiol 2001;127:240–51. https://doi.org/10.1104/pp.127.1.240.
[88] Shiva S, Samarakoon T, Lowe KA, Roach C, Vu HS, Colter M, et al. Leaf lipid alterations in response to heat stress of Arabidopsis thaliana. Plants 2020;9:845. https://doi.org/10.3390/plants9070845.
[89] Blancaflor EB, Kilaru A, Keereetaweep J, Khan BR, Faure L, Chapman KD. N-Acylethanolamines: lipid metabolites with functions in plant growth and development. Plant J 2014;79:568–83. https://doi.org/10.1111/tpj.12427.
[90] Vu HS, Roth MR, Tamura P, Samarakoon T, Shiva S, Honey S, et al. Head-group acylation of monogalactosyldiacylglycerol is a common stress response, and the acyl-galactose acyl composition varies with the plant species and applied stress. Physiol Plant 2014;150:517–28. https://doi.org/10.1111/ppl.12132.
[91] Al-Fadhli A, Wahidulla S, D'Souza L. Glycolipids from the red aAlga Chondria armata (Kütz.) Okamura. Glycobiology 2006;16:902–15. https://doi.org/10.1093/glycob/cwl018.
[92] Wang H, Yang L, Wang X, Cong P, Xu J, Xue C. Comprehensive lipidomic analysis of three edible brown seaweeds based on reversed-phase liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. J Agric Food Chem 2022;70:4138–51. https://doi.org/10.1021/acs.jafc.1c07513.
[93] Holmback J, Karlsson A, Arnoldsson KC. Characterization of N-acylphosphatidylethanolamine and acylphosphatidylglycerol in oats. Lipids 2001;36:153–65. https://doi.org/10.1007/s11745-001-0702-z.
[94] Nilsson AK, Johansson ON, Fahlberg P, Steinhart F, Gustavsson MB, Ellerström M, et al. Formation of oxidized phosphatidylinositol and 12-oxo-phytodienoic acid containing acylated phosphatidylglycerol during the hypersensitive response in Arabidopsis. Phytochemistry 2014;101:65–75. https://doi.org/10.1016/j.phytochem.2014.01.020.
[95] Mueller SP, Unger M, Guender L, Fekete A, Mueller MJ. Phospholipid: diacylglycerol acyltransferase-mediated triacylglyerol synthesis augments basal thermotolerance. Plant Physiol 2017;175:486–97. https://doi.org/10.1104/pp.17.00861.
[96] Nilsson AK, Johansson ON, Fahlberg P, Kommuri M, Töpel M, Bodin LJ, et al. Acylated monogalactosyl diacylglycerol: prevalence in the plant kingdom and identification of an enzyme catalyzing galactolipid head group acylation in *Arabidopsis thaliana*. Plant J 2015;84:1152–66. https://doi.org/10.1111/tpj.13072.
[97] Lupette J, Maréchal E. Phytoplankton glycerolipids: challenging but promising prospects from biomedicine to green chemistry and biofuels. Blue biotechnology. John Wiley & Sons, Ltd; 2018. p. 191–215. https://doi.org/10.1002/9783527801718.ch6.
[98] Chandler IC. Determining the regioselectivity of immobilized lipases in triacylglycerol acidolysis reactions. J Am Oil Chem Soc 2001;78:737–42. https://doi.org/10.1007/s11746-001-0335-7.
[99] Vanhercke T, Dyer JM, Mullen RT, Kilaru A, Rahman MM, Petrie JR, et al. Metabolic engineering for enhanced oil in biomass. Prog Lipid Res 2019;74:103–29. https://doi.org/10.1016/j.plipres.2019.02.002.
[100] Nam J-W, Lee HG, Do H, Kim HU, Seo PJ. Transcriptional regulation of triacylglycerol accumulation in plants under environmental stress conditions. J Exp Bot 2022;73:2905–17. https://doi.org/10.1093/jxb/erab554.
[101] Jackson CL. Lipid droplet biogenesis. Curr Opin Cell Biol 2019;59:88–96. https://doi.org/10.1016/j.ceb.2019.03.018.
[102] Nguyen HM, Baudet M, Cuiné S, Adriano J-M, Barthe D, Billon E, et al. Proteomic profiling of oil bodies isolated from the unicellular green microalga *Chlamydomonas reinhardtii*: with focus on proteins involved in lipid metabolism. PROTEOMICS 2011;11:4266–73. https://doi.org/10.1002/pmic.201100114.
[103] Jaussaud A, Lupette J, Salvaing J, Jouhet J, Bastien O, Gromova M, et al. Stepwise biogenesis of subpopulations of lipid droplets in nitrogen starved *Phaeodactylum tricornutum* cells. Front Plant Sci 2020:11.
[104] Lupette J, Jaussaud A, Seddiki K, Morabito C, Brugière S, Schaller H, et al. The architecture of lipid droplets in the diatom *Phaeodactylum tricornutum*. Algal Res 2019;38:101415. https://doi.org/10.1016/j.algal.2019.101415.
[105] Popko J, Herrfurth C, Feussner K, Ischebeck T, Iven T, Haslam R, et al. Metabolome analysis reveals betaine Lipids as major source for triglyceride formation, and the accumulation of Sedoheptulose during nitrogen-starvation of *Phaeodactylum tricornutum*. PLoS One 2016;11:e0164673. https://doi.org/10.1371/journal.pone.0164673.
[106] Alboresi A, Perin G, Vitulo N, Diretto G, Block M, Jouhet J, et al. Light remodels lipid biosynthesis in nannochloropsis gaditana by modulating carbon partitioning between organelles. Plant Physiol 2016;171:2468–82. https://doi.org/10.1104/pp.16.00599.
[107] Dellero Y, Cagnac O, Rose S, Seddiki K, Cussac M, Morabito C, et al. Proposal of a new thraustochytrid genus Hondaea gen. Nov. and comparison of its lipid dynamics with the closely related pseudo-cryptic genus Aurantiochytrium. Algal Res 2018;35:125–41. https://doi.org/10.1016/j.algal.2018.08.018.
[108] Lupette J, Benning C. Human health benefits of very-long-chain polyunsaturated fatty acids from microalgae. Biochimie 2020;178:15–25. https://doi.org/10.1016/j.biochi.2020.04.022.
[109] Kehelpannala C, Rupasinghe T, Pasha A, Esteban E, Hennessy T, Bradley D, et al. An Arabidopsis lipid map reveals differences between tissues and dynamic changes throughout development. Plant J 2021;107:287–302. https://doi.org/10.1111/tpj.15278.
[110] Da Costa E, Azevedo V, Melo T, Rego AM, Evtuguin V, Domingues P, et al. High-resolution Lipidomics of the early life stages of the red seaweed *Porphyra dioica*. Molecules 2018;23:187. https://doi.org/10.3390/molecules23010187.
[111] Babiychuk E, Bouvier-Navé P, Compagnon V, Suzuki M, Muranaka T, Van Montagu M, et al. Allelic mutant series reveal distinct functions for Arabidopsis cycloartenol synthase 1 in cell viability and plastid biogenesis. Proc Natl Acad Sci USA 2008;105:3163–8. https://doi.org/10.1073/pnas.0712190105.
[112] Nes WD. Biosynthesis of cholesterol and other sterols. Chem Rev 2011;111:6423–51. https://doi.org/10.1021/cr200021m.
[113] Nakamoto M, Schmit A-C, Heintz D, Schaller H, Ohta D. Diversification of sterol methyltransferase enzymes in plants and a role for β-sitosterol in oriented cell plate formation and polarized growth. Plant J 2015;84:860–74. https://doi.org/10.1111/tpj.13043.
[114] Rogowska A, Szakiel A. The role of sterols in plant response to abiotic stress. Phytochem Rev 2020;19:1525–38. https://doi.org/10.1007/s11101-020-09708-2.
[115] Seitz LM. Stanol and sterol esters of ferulic and p-coumaric acids in wheat, corn, rye, and triticale. J Agric Food Chem 1989;37:662–7. https://doi.org/10.1021/jf00087a019.
[116] Ito J, Sawada K, Ogura Y, Xinyi F, Rahmania H, Mohri T, et al. Definitive evidence of the presence of 24-methylenecycloartanyl ferulate and 24-methylenecycloartanyl caffeate in barley. Sci Rep 2019;9:12572. https://doi.org/10.1038/s41598-019-48985-6.
[117] Ferrer A, Altabella T, Arró M, Boronat A. Emerging roles for conjugated sterols in plants. Prog Lipid Res 2017;67:27–37. https://doi.org/10.1016/j.plipres.2017.06.002.
[118] Darnet S, Blary A, Chevalier Q, Schaller H. Phytosterol profiles, genomes and enzymes – an overview. Front Plant Sci 2021:12. https://doi.org/10.3389/fpls.2021.665206.
[119] Sawai S, Ohyama K, Yasumoto S, Seki H, Sakuma T, Yamamoto T, et al. Sterol side chain reductase 2 is a key enzyme in the biosynthesis of cholesterol, the common precursor of toxic steroidal glycoalkaloids in potato. Plant Cell 2014;26:3763–74. https://doi.org/10.1105/tpc.114.130096.
[120] Hambleton EA, Jones VAS, Maegele I, Kvaskoff D, Sachsenheimer T, Guse A. Sterol transfer by atypical cholesterol-binding NPC2 proteins in coral-algal symbiosis. ELife 2019;8:e43923. https://doi.org/10.7554/eLife.43923.
[121] Giner JLuis.. Biosynthesis of marine sterol side chains. Chem Rev 1993;93:1735–52. https://doi.org/10.1021/cr00021a004.
[122] Girard J, Lanneau G, Delage L, Leroux C, Belcour A, Got J, et al. Semi-quantitative targeted gas chromatography-mass spectrometry profiling supports a late side-chain reductase Cycloartenol-to-cholesterol biosynthesis pathway in Brown algae. Front Plant Sci 2021:12. https://doi.org/10.3389/fpls.2021.648426.
[123] Schrick K, Shiva S, Arpin JC, Delimont N, Isaac G, Tamura P, et al. Steryl glucoside and acyl steryl glucoside analysis of Arabidopsis seeds by electrospray ionization tandem mass spectrometry. Lipids 2012;47:185–93. https://doi.org/10.1007/s11745-011-3602-9.
[124] Furse S, Martel C, Yusuf A, Shearman GC, Koch H, Stevenson PC. Sterol composition in plants is specific to pollen, leaf, pollination and pollinator. Phytochemistry 2023;214:113800. https://doi.org/10.1016/j.phytochem.2023.113800.
[125] Wewer V, Dombrink I, vom Dorp K, Dörmann P. Quantification of sterol lipids in plants by quadrupole time-of-flight mass spectrometry. J Lipid Res 2011;52:1039–54. https://doi.org/10.1194/jlr.D013987.
[126] DeBolt S, Scheible W-R, Schrick K, Auer M, Beisson F, Bischoff V, et al. Mutations in UDP-glucose:sterol glucosyltransferase in arabidopsis cause transparent testa phenotype and suberization defect in seeds. Plant Physiol 2009;151:78–87. https://doi.org/10.1104/pp.109.140582.
[127] Michaelson LV, Napier JA, Molino D, Faure J-D. Plant sphingolipids: their importance in cellular organization and adaption. Biochim Biophys Acta (BBA) -

Mol Cell Biol Lipids 2016;1861:1329–35. https://doi.org/10.1016/j.bbalip.2016.04.003.
[128] Huby E, Napier JA, Baillieul F, Michaelson LV, Dhondt-Cordelier S. Sphingolipids: towards an integrated view of metabolism during the plant stress response. New Phytol 2020;225:659–70. https://doi.org/10.1111/nph.15997.
[129] Markham JE, Jaworski JG. Rapid measurement of sphingolipids from Arabidopsis thaliana by reversed-phase high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 2007;21:1304–14. https://doi.org/10.1002/rcm.2962.
[130] Cacas J-L, Furt F, Le Guédard M, Schmitter J-M, Buré C, Gerbeau-Pissot P, et al. Lipids of plant membrane rafts. Prog Lipid Res 2012;51:272–99. https://doi.org/10.1016/j.plipres.2012.04.001.
[131] Ziv C, Malitsky S, Othman A, Ben-Dor S, Wei Y, Zheng S, et al. Viral serine palmitoyltransferase induces metabolic switch in sphingolipid biosynthesis and is required for infection of a marine alga. Proc Natl Acad Sci USA 2016;113:E1907–16. https://doi.org/10.1073/pnas.1523168113.
[132] Li Y, Lou Y, Mu T, Ke A, Ran Z, Xu J, et al. Sphingolipids in marine microalgae: development and application of a mass spectrometric method for global structural characterization of ceramides and glycosphingolipids in three major phyla. Anal Chim Acta 2017;986:82–94. https://doi.org/10.1016/j.aca.2017.07.039.
[133] Imai H, Yamamoto K, Shibahara A, Miyatani S, Nakayama T. Determining double-bond positions in monoenoic 2-hydroxy fatty acids of glucosylceramides by gas chromatography-mass spectrometry. Lipids 2000;35:233–6. https://doi.org/10.1007/BF02664774.
[134] Markham JE, Li J, Cahoon EB, Jaworski JG. Separation and identification of major plant sphingolipid classes from leaves. J Biol Chem 2006;281:22684–94. https://doi.org/10.1074/jbc.M604050200.
[135] König S, Feussner K, Schwarz M, Kaever A, Iven T, Landesfeind M, et al. Arabidopsis mutants of sphingolipid fatty acid α-hydroxylases accumulate ceramides and salicylates. New Phytol 2012;196:1086–97. https://doi.org/10.1111/j.1469-8137.2012.04351.x.
[136] Sperling P, Franke S, Lüthje S, Heinz E. Are glucocerebrosides the predominant sphingolipids in plant plasma membranes? Plant Physiol Biochem 2005;43:1031–8. https://doi.org/10.1016/j.plaphy.2005.10.004.
[137] Luttgeharm KD, Chen M, Mehra A, Cahoon RE, Markham JE, Cahoon EB. Overexpression of arabidopsis ceramide synthases differentially affects growth, sphingolipid metabolism, programmed cell death, and mycotoxin resistance. Plant Physiol 2015;169:1108–17. https://doi.org/10.1104/pp.15.00987.
[138] Stonik VA, Stonik IV. Sterol and sphingoid glycoconjugates from microalgae. Mar Drugs 2018;16:514. https://doi.org/10.3390/md16120514.
[139] Lowenstein DP, Mayers K, Fredricks HF, Van Mooy BAS. Targeted and untargeted lipidomic analysis of haptophyte cultures reveals novel and divergent nutrient-stress adaptations. Org Geochem 2021;161:104315. https://doi.org/10.1016/j.orggeochem.2021.104315.
[140] Malitsky S, Ziv C, Rosenwasser S, Zheng S, Schatz D, Porat Z, et al. Viral infection of the marine alga *Emiliania huxleyi* triggers lipidome remodeling and induces the production of highly saturated triacylglycerol. New Phytol 2016;210:88–96. https://doi.org/10.1111/nph.13852.
[141] Melo T, Alves E, Azevedo V, Martins AS, Neves B, Domingues P, et al. Lipidomics as a new approach for the bioprospecting of marine macroalgae — unraveling the polar lipid and fatty acid composition of *Chondrus crispus*. Algal Res 2015;8:181–91. https://doi.org/10.1016/j.algal.2015.02.016.
[142] Vítová M, Čížková M, Náhlík V, Řezanka T. Changes in glycosyl inositol phosphoceramides during the cell cycle of the red alga Galdieria sulphuraria. Phytochemistry 2022;194:113025. https://doi.org/10.1016/j.phytochem.2021.113025.
[143] Vardi A, Van Mooy BAS, Fredricks HF, Popendorf KJ, Ossolinski JE, Haramaty L, et al. Viral glycosphingolipids induce lytic infection and cell death in marine phytoplankton. Science 2009;326:861–5. https://doi.org/10.1126/science.1177322.
[144] Bahammou D, Recorbet G, Mamode Cassim A, Robert F, Balliau T, Van Delft P, et al. A combined lipidomic and proteomic profiling of *Arabidopsis thaliana* plasma membrane. Plant J n.d.;n/a. doi:https://doi.org/10.1111/tpj.16810.
[145] Cacas J-L, Buré C, Grosjean K, Gerbeau-Pissot P, Lherminier J, Rombouts Y, et al. Revisiting plant plasma membrane Lipids in tobacco: A focus on Sphingolipids1. Plant Physiol 2016;170:367–84. https://doi.org/10.1104/pp.15.00564.
[146] Ali U, Li H, Wang X, Guo L. Emerging roles of sphingolipid signaling in plant response to biotic and abiotic stresses. Mol Plant 2018;11:1328–43. https://doi.org/10.1016/j.molp.2018.10.001.
[147] Xie L-J, Chen Q-F, Chen M-X, Yu L-J, Huang L, Chen L, et al. Unsaturation of very-long-chain ceramides protects plant from hypoxia-induced damages by modulating ethylene signaling in Arabidopsis. PLoS Genet 2015;11:e1005143. https://doi.org/10.1371/journal.pgen.1005143.
[148] Takahashi D, Imai H, Kawamura Y, Uemura M. Lipid profiles of detergent resistant fractions of the plasma membrane in oat and rye in association with cold acclimation and freezing tolerance. Cryobiology 2016;72:123–34. https://doi.org/10.1016/j.cryobiol.2016.02.003.
[149] Zhou Y, Hölzl G, vom Dorp K, Peisker H, Melzer M, Frentzen M, et al. Identification and characterization of a plastidial phosphatidylglycerophosphate phosphatase in *Arabidopsis thaliana*. Plant J 2017;89:221–34. https://doi.org/10.1111/tpj.13378.
[150] Molino D, Van der Giessen E, Gissot L, Hématy K, Marion J, Barthelemy J, et al. Inhibition of very long acyl chain sphingolipid synthesis modifies membrane dynamics during plant cytokinesis. Biochim Biophys Acta (BBA) - Mol Cell Biol Lipids 2014;1841:1422–30. https://doi.org/10.1016/j.bbalip.2014.06.014.
[151] Yanagawa D, Ishikawa T, Imai H. Synthesis and degradation of long-chain base phosphates affect fumonisin B1-induced cell death in Arabidopsis thaliana. J Plant Res 2017;130:571–85. https://doi.org/10.1007/s10265-017-0923-7.
[152] Lenarčič T, Albert I, Böhm H, Hodnik V, Pirc K, Zavec AB, et al. Eudicot plant-specific sphingolipids determine host selectivity of microbial NLP cytolysins. Science 2017;358:1431–4. https://doi.org/10.1126/science.aan6874.
[153] Fulton JM, Fredricks HF, Bidle KD, Vardi A, Kendrick BJ, DiTullio GR, et al. Novel molecular determinants of viral susceptibility and resistance in the lipidome of Emiliania huxleyi. Environ Microbiol 2014;16:1137–49. https://doi.org/10.1111/1462-2920.12358.
[154] Schleyer G, Shahaf N, Ziv C, Dong Y, Meoded RA, Helfrich EJN, et al. In plaque-mass spectrometry imaging of a bloom-forming alga during viral infection reveals a metabolic shift towards odd-chain fatty acid lipids. Nat Microbiol 2019;4:527–38. https://doi.org/10.1038/s41564-018-0336-y.
[155] Schleyer G, Kuhlisch C, Ziv C, Ben-Dor S, Malitsky S, Schatz D, et al. Lipid biomarkers for algal resistance to viral infection in the ocean. Proc Natl Acad Sci 2023;120:e2217121120. https://doi.org/10.1073/pnas.2217121120.
[156] Haynes CA, Allegood JC, Park H, Sullards MC. Sphingolipidomics: methods for the comprehensive analysis of sphingolipids. J Chromatogr B 2009;877:2696–708. https://doi.org/10.1016/j.jchromb.2008.12.057.
[157] Jahn U, Galano J-M, Durand T. Beyond prostaglandins—chemistry and biology of cyclic oxygenated metabolites formed by free-radical pathways from polyunsaturated fatty acids. Angew Chem Int Ed 2008;47:5894–955. https://doi.org/10.1002/anie.200705122.
[158] Hajeyah AA, Griffiths WJ, Wang Y, Finch AJ, O'Donnell VB. The biosynthesis of enzymatically oxidized Lipids. Front Endocrinol 2020;11.
[159] Yu D, Boughton BA, Hill CB, Feussner I, Roessner U, Rupasinghe TWT. Insights into oxidized lipid modification in barley roots as an adaptation mechanism to salinity stress. Front Plant Sci 2020;11:1. https://doi.org/10.3389/fpls.2020.00001.
[160] Conde TA, Couto D, Melo T, Costa M, Silva J, Domingues MR, et al. Polar lipidomic profile shows Chlorococcum amblystomatis as a promising source of value-added lipids. Sci Rep 2021;11:1–23. https://doi.org/10.1038/s41598-021-83455-y.
[161] Moreira ASP, Gonçalves J, Conde TA, Couto D, Melo T, Maia IB, et al. Chrysotila pseudoroscoffensis as a source of high-value polar lipids with antioxidant activity: A lipidomic approach. Algal Res 2022;66:102756. https://doi.org/10.1016/j.algal.2022.102756.
[162] Mori N, Moriyama T, Toyoshima M, Sato N. Construction of global acyl lipid metabolic map by comparative genomics and subcellular localization analysis in the red alga Cyanidioschyzon merolae. Front Plant Sci 2016:7.
[163] Vítová M, Goecke F, Sigler K, Řezanka T. Lipidomic analysis of the extremophilic red alga Galdieria sulphuraria in response to changes in pH. Algal Res 2016;13:218–26. https://doi.org/10.1016/j.algal.2015.12.005.
[164] da Costa E, Melo T, Moreira ASP, Alves E, Domingues P, Calado R, et al. Decoding bioactive polar lipid profile of the macroalgae *Codium tomentosum* from a sustainable IMTA system using a lipidomic approach. Algal Res 2015;12:388–97. https://doi.org/10.1016/j.algal.2015.09.020.
[165] Jagusch H, Werner M, Werz O, Pohnert G. 15-Hydroperoxy-PGE2: intermediate in mammalian and algal prostaglandin biosynthesis. Angew Chem Int Ed 2019;58:17641–5. https://doi.org/10.1002/anie.201910461.
[166] Goulah CC, Zhu G, Koszelak-Rosenblum M, Malkowski MG. The crystal structure of α-dioxygenase provides insight into diversity in the cyclooxygenase-peroxidase superfamily. Biochemistry 2013;52:1364–72. https://doi.org/10.1021/bi400013k.
[167] Wasternack C, Feussner I. The oxylipin pathways: biochemistry and function. Annu Rev Plant Biol 2018;69:363–86. https://doi.org/10.1146/annurev-arplant-042817-040440.
[168] Mueller MJ, Mène-Saffrané L, Grun C, Karg K, Farmer EE. Oxylipin analysis methods. Plant J 2006;45:472–89. https://doi.org/10.1111/j.1365-313X.2005.02614.x.
[169] Andreou A, Brodhun F, Feussner I. Biosynthesis of oxylipins in non-mammals. Prog Lipid Res 2009;48:148–70. https://doi.org/10.1016/j.plipres.2009.02.002.
[170] Viswanath KK, Varakumar P, Pamuru RR, Basha SJ, Mehta S, Rao AD. Plant lipoxygenases and their role in plant physiology. J Plant Biol 2020;63:83–95. https://doi.org/10.1007/s12374-020-09241-x.
[171] Griffiths G. Biosynthesis and analysis of plant oxylipins. Free Radic Res 2015;49:565–82. https://doi.org/10.3109/10715762.2014.1000318.
[172] Gerwick WH, Proteau PJ, Nagle DG, Wise ML, Jiang ZD, Bernart MW, et al. Biologically active oxylipins from seaweeds. Hydrobiologia 1993;260:653–65. https://doi.org/10.1007/BF00049085.
[173] Barbosa M, Valentão P, Andrade PB. Biologically active Oxylipins from enzymatic and nonenzymatic routes in macroalgae. Mar Drugs 2016;14:23–49. https://doi.org/10.3390/md14010023.
[174] Lupette J, Jaussaud A, Vigor C, Oger C, Galano J-M, Réversat G, et al. Non-enzymatic synthesis of bioactive Isoprostanoids in the diatom *Phaeodactylum* following oxidative stress. Plant Physiol 2018;178:1344–57. https://doi.org/10.1104/pp.18.00925.
[175] Huang H, Liu B, Liu L, Song S. Jasmonate action in plant growth and development. J Exp Bot 2017;68:1349–59. https://doi.org/10.1093/jxb/erw495.
[176] Monte I, Ishida S, Zamarreño AM, Hamberg M, Franco-Zorrilla JM, García-Casado G, et al. Ligand-receptor co-evolution shaped the jasmonate pathway in land plants. Nat Chem Biol 2018;14:480–8. https://doi.org/10.1038/s41589-018-0033-4.

[177] Krupina MV, Dathe W. Occurrence of Jasmonic acid in the red alga Gelidium latifolium. Zeitsch Naturforsch C 1991;46:1127–9. https://doi.org/10.1515/znc-1991-11-1230.

[178] Junichi U, Kensuke M, Miyako A, Tsuyoshi H, Tomohiro S, Yoshihide M. Identification of Jasmonic acid in Chlorella and Spirulina. Bull Univer Osaka Prefect Ser B, Agr Biol 1991;43:103–8. https://doi.org/10.24729/00009274.

[179] Fujii S, Yamamoto R, Miyamoto K, Ueda J. Occurrence of jasmonic acid in Dunaliella (Dunaliellales, Chlorophyta). Phycol Res 1997;45:223–6. https://doi.org/10.1111/j.1440-1835.1997.tb00080.x.

[180] Weinberger F, Lion U, Delage L, Kloareg B, Potin P, Beltrán J, et al. Up-regulation of lipoxygenase, phospholipase, and Oxylipin-production in the induced chemical defense of the red alga Gracilaria chilensis against epiphytes. J Chem Ecol 2011; 37:677–86. https://doi.org/10.1007/s10886-011-9981-9.

[181] Nilsson AK, Fahlberg P, Ellerström M, Andersson MX. Oxo-phytodienoic acid (OPDA) is formed on fatty acids esterified to galactolipids after tissue disruption in *Arabidopsis thaliana*. FEBS Lett 2012;586:2483–7. https://doi.org/10.1016/j.febslet.2012.06.010.

[182] Fessel JP, Porter NA, Moore KP, Sheller JR, Roberts LJ. Discovery of lipid peroxidation products formed in vivo with a substituted tetrahydrofuran ring (isofurans) that are favored by increased oxygen tension. Proc Natl Acad Sci 2002; 99:16713–8. https://doi.org/10.1073/pnas.252649099.

[183] Leung KS, Oger C, Guy A, Bultel-Poncé V, Vigor C, Durand T, et al. Alpha-linolenic acid, phytoprostanes and phytofurans in plant, algae and food. Adv Bot Res 2022; 101:437–68. https://doi.org/10.1016/bs.abr.2021.09.005. Elsevier.

[184] Barbosa M, Collado-González J, Andrade PB, Ferreres F, Valentão P, Galano J-M, et al. Nonenzymatic α-linolenic acid derivatives from the sea: macroalgae as novel sources of phytoprostanes. J Agric Food Chem 2015;63:6466–74. https://doi.org/10.1021/acs.jafc.5b01904.

[185] Loeffler C, Berger S, Guy A, Durand T, Bringmann G, Dreyer M, et al. B1-phytoprostanes trigger plant defense and detoxification responses. Plant Physiol 2005;137:328–40. https://doi.org/10.1104/pp.104.051714.

[186] Imbusch R, Mueller MJ. Analysis of oxidative stress and wound-inducible Dinor Isoprostanes F1 (Phytoprostanes F1) in plants. Plant Physiol 2000;124:1293–304. https://doi.org/10.1104/pp.124.3.1293.

[187] González Roldán N, Engel R, Düpow S, Jakob K, Koops F, Orinska Z, et al. Lipid mediators from Timothy grass pollen contribute to the effector phase of allergy and prime dendritic cells for glycolipid presentation. Front Immunol 2019;10: 974. https://doi.org/10.3389/fimmu.2019.00974.

[188] Yonny ME, Rodríguez Torresi A, Cuyamendous C, Réversat G, Oger C, Galano J-M, et al. Thermal stress in melon plants: Phytoprostanes and Phytofurans as oxidative stress biomarkers and the effect of antioxidant supplementation. J Agric Food Chem 2016;64:8296–304. https://doi.org/10.1021/acs.jafc.6b03011.

[189] Rac M, Shumbe L, Oger C, Guy A, Vigor C, Ksas B, et al. Luminescence imaging of leaf damage induced by lipid peroxidation products and its modulation by β-cyclocitral. Physiol Plant 2021;171:246–59. https://doi.org/10.1111/ppl.13279.

[190] Cuyamendous C, Leung KS, Durand T, Lee JC-Y, Oger C, Galano J-M. Synthesis and discovery of phytofurans: metabolites of α-linolenic acid peroxidation. Chem Commun 2015;51:15696–9. https://doi.org/10.1039/C5CC05736A.

[191] Cuyamendous C, Leung KS, Bultel-Poncé V, Guy A, Durand T, Galano J-M, et al. Total synthesis and in vivo quantitation of phytofurans derived from α-linolenic acid. Eur J Org Chem 2017;2017:2486–90. https://doi.org/10.1002/ejoc.201700270.

[192] Vigor C, Reversat G, Rocher A, Oger C, Galano J-M, Vercauteren J, et al. Isoprostanoids quantitative profiling of marine red and brown macroalgae. Food Chem 2018;268:452–62. https://doi.org/10.1016/j.foodchem.2018.06.111.

[193] Dupuy A, Le Faouder P, Vigor C, Galano J-M, Dray C, Chung-Yung Lee J, et al. Simultaneous quantitative profiling of 20 isoprostanoids from omega-3 and omega-6 polyunsaturated fatty acids by LC-MS/MS in various biological samples. Anal Chim Acta 2016;921:46–58. https://doi.org/10.1016/j.aca.2016.03.024.

[194] Kitano S, Hisatomi H, Hibi N, Kawano K, Harada S. Improved method of plasma 8-Isoprostane measurement and association analyses with habitual drinking and smoking. World J Gastroenterol 2006;12:5846–52. https://doi.org/10.3748/wjg.v12.i36.5846.

[195] Södergren E, Vessby B, Basu S. Radioimmunological measurement of F2-isoprostanes after hydrolysis of lipids in tissues. Prostagland Leukotrien Essent Fatty Acids (PLEFA) 2000;63:149–52. https://doi.org/10.1054/plef.2000.0172.

[196] Tsikas D. Application of gas chromatography–mass spectrometry and gas chromatography–tandem mass spectrometry to assess in vivo synthesis of prostaglandins, thromboxane, leukotrienes, isoprostanes and related compounds in humans1Dedicated to Prof. Jürgen C. Frölich for his 60th anniversary 1. J Chromatogr B Biomed Sci Appl 1998;717:201–45. https://doi.org/10.1016/S0378-4347(98)00210-2.

[197] Melo T, Montero-Bullón J-F, Domingues P, Domingues MR. Discovery of bioactive nitrated lipids and nitro-lipid-protein adducts using mass spectrometry-based approaches. Redox Biol 2019:1–16. https://doi.org/10.1016/j.redox.2019.101106.

[198] Mata-Pérez C, Sánchez-Calvo B, Padilla MN, Begara-Morales JC, Valderrama R, Corpas FJ, et al. Nitro-fatty acids in plant signaling: new key mediators of nitric oxide metabolism. Redox Biol 2017;11:554–61. https://doi.org/10.1016/j.redox.2017.01.002.

[199] Mata-Pérez C, Sánchez-Calvo B, Padilla MN, Begara-Morales JC, Luque F, Melguizo M, et al. Nitro-fatty acids in plant signaling: nitro-linolenic acid induces the molecular chaperone network in Arabidopsis. Plant Physiol 2016;170: 686–701. https://doi.org/10.1104/pp.15.01671.

[200] Mata-Pérez C, Sánchez-Calvo B, Padilla MN, Begara-Morales JC, Valderrama R, Corpas FJ, et al. Nitro-fatty acids in plant signaling: new key mediators of nitric oxide metabolism. Redox Biol 2017;11:554–61. https://doi.org/10.1016/j.redox.2017.01.002.

[201] Fazzari M, Trostchansky A, Schopfer FJ, Salvatore SR, Sánchez-Calvo B, Vitturi D, et al. Olives and olive oil are sources of electrophilic fatty acid nitroalkenes. PLoS One 2014;9:1–9. https://doi.org/10.1371/journal.pone.0084884.

[202] Vollár M, Feigl G, Oláh D, Horváth A, Molnár Á, Kúsz N, et al. Nitro-oleic acid in seeds and differently developed seedlings of *Brassica napus* L. Plants 2020;9:1–15. https://doi.org/10.3390/plants9030406.

[203] Di Fino L, Arruebarrena Di Palma A, Perk EA, García-Mata C, Schopfer FJ, Laxalt AM. Nitro-fatty acids: electrophilic signaling molecules in plant physiology. Planta 2021;254:120. https://doi.org/10.1007/s00425-021-03777-z.

[204] Lopes D, Moreira ASP, Rey F, da Costa E, Melo T, Maciel E, et al. Lipidomic signature of the green macroalgae Ulva rigida farmed in a sustainable integrated multi-trophic aquaculture. J Appl Phycol 2019;31:1369–81. https://doi.org/10.1007/s10811-018-1644-6.

[205] Moreira ASP, da Costa E, Melo T, Lopes D, Pais ACS, Santos SAO, et al. Polar Lipids of commercial Ulva spp. of different origins: profiling and relevance for seaweed valorization. Foods 2021;10:914. https://doi.org/10.3390/foods10050914.

[206] Monteiro JP, Rey F, Melo T, Moreira ASP, Arbona J-F, Skjermo J, et al. The unique Lipidomic signatures of *Saccharina latissima* can be used to pinpoint their geographic origin. Biomolecules 2020;10:E107. https://doi.org/10.3390/biom10010107.

[207] da Costa E, Ricardo F, Melo T, Mamede R, Abreu MH, Domingues P, et al. Site-specific Lipidomic signatures of sea lettuce (Ulva spp., Chlorophyta) hold the potential to trace their geographic origin. Biomolecules 2020;10:489. https://doi.org/10.3390/biom10030489.

[208] Rey F, Cartaxana P, Melo T, Calado R, Pereira R, Abreu H, et al. Domesticated populations of *Codium tomentosum* display lipid extracts with lower seasonal shifts than conspecifics from the wild—relevance for biotechnological applications of this green seaweed. Mar Drugs 2020;18:188. https://doi.org/10.3390/md18040188.

[209] Couto D, Conde TA, Melo T, Neves B, Costa M, Cunha P, et al. Effects of outdoor and indoor cultivation on the polar lipid composition and antioxidant activity of Nannochloropsis oceanica and Nannochloropsis limnetica: a lipidomics perspective. Algal Res 2022;64:102718. https://doi.org/10.1016/j.algal.2022.102718.

[210] Ryu SB, Wang X. Activation of phospholipase D and the possible mechanism of activation in wound-induced lipid hydrolysis in castor bean leaves. Biochim Biophys Acta (BBA) - Lipids Lipid Metabol 1996;1303:243–50. https://doi.org/10.1016/0005-2760(96)00096-3.

[211] Chen L, Liu T, Zhang W, Chen X, Wang J. Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. Bioresour Technol 2012;111: 208–14. https://doi.org/10.1016/j.biortech.2012.02.033.

[212] Singh G, Patidar SK. Microalgae harvesting techniques: A review. J Environ Manag 2018;217:499–508. https://doi.org/10.1016/j.jenvman.2018.04.010.

[213] Barouh N, Wind J, Chuat V, Gagnaire V, Valence F, Bourlieu-Lacanal C, et al. Variations in Chlorella lipid content in commercial and in-lab produced biomass. OCL 2024;31:9. https://doi.org/10.1051/ocl/2024005.

[214] Kates M. Effects of solvents and surface-active agents on plastid phosphatidase C activity. Can J Biochem Physiol 1957;35:127–42.

[215] Bengtsson JD, Wallis JG, Browse J. Lipid isolation from plants. In: Bartels D, Dörmann P, editors. Plant Lipids: Methods and protocols. New York, NY: Springer US; 2021. p. 3–13. https://doi.org/10.1007/978-1-0716-1362-7_1.

[216] Cikoš A-M, Jokić S, Šubarić D, Jerković I. Overview on the application of modern methods for the extraction of bioactive compounds from marine macroalgae. Mar Drugs 2018;16:348. https://doi.org/10.3390/md16100348.

[217] Ryckebosch E, Bermúdez SPC, Termote-Verhalle R, Bruneel C, Muylaert K, Parra-Saldivar R, et al. Influence of extraction solvent system on the extractability of lipid components from the biomass of *Nannochloropsis gaditana*. J Appl Phycol 2014;26:1501–10. https://doi.org/10.1007/s10811-013-0189-y.

[218] Kehelpannala C, Rupasinghe TWT, Hennessy T, Bradley D, Ebert B, Roessner U. A comprehensive comparison of four methods for extracting lipids from Arabidopsis tissues. Plant Methods 2020;16:155. https://doi.org/10.1186/s13007-020-00697-z.

[219] Ummat V, Sivagnanam SP, Rajauria G, O'Donnell C, Tiwari BK. Advances in pre-treatment techniques and green extraction technologies for bioactives from seaweeds. Trends Food Sci Technol 2021;110:90–106. https://doi.org/10.1016/j.tifs.2021.01.018.

[220] Servaes K, Maesen M, Prandi B, Sforza S, Elst K. Polar lipid profile of Nannochloropsis oculata determined using a variety of lipid extraction procedures. J Agric Food Chem 2015;63:3931–41. https://doi.org/10.1021/acs.jafc.5b00241.

[221] Folch J, Ascoli I, Lees M, Meath JA, LeBARON N. Preparation of lipide extracts from brain tissue. J Biol Chem 1951;191:833–41.

[222] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7. https://doi.org/10.1139/o59-099.

[223] Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J Lipid Res 2008;49:1137–46. https://doi.org/10.1194/jlr.D700041-JLR200.

[224] Ryckebosch E, Bruneel C, Termote-Verhalle R, Muylaert K, Foubert I. Influence of extraction solvent system on extractability of lipid components from different microalgae species. Algal Res 2014;3:36–43. https://doi.org/10.1016/j.algal.2013.11.001.

[225] Jiménez Callejón MJ, Robles Medina A, Macías Sánchez MD, Esteban Cerdán L, González Moreno PA, Navarro López E, et al. Obtaining highly pure EPA-rich lipids from dry and wet Nannochloropsis gaditana microalgal biomass using ethanol, hexane and acetone. Algal Res 2020;45:101729. https://doi.org/10.1016/j.algal.2019.101729.
[226] Mubarak M, Shaija A, Suchithra TV. A review on the extraction of lipid from microalgae for biodiesel production. Algal Res 2015;7:117–23. https://doi.org/10.1016/j.algal.2014.10.008.
[227] Watanabe A, Balas L, Saigusa D, Ogura J, Durand T, Mano N, et al. Analytical evaluation of fatty acid esters of hydroxy fatty acid quantity in fermented brown rice and rice bran (FRBA). Food Chem Adv 2022;1:100040. https://doi.org/10.1016/j.focha.2022.100040.
[228] Da Costa E, Melo T, Moreira ASP, Bernardo C, Helguero L, Ferreira I, et al. Valorization of Lipids from Gracilaria sp. through Lipidomics and decoding of Antiproliferative and anti-inflammatory activity. Mar Drugs 2017;15:62. https://doi.org/10.3390/md15030062.
[229] Rizov I, Doulis A. Separation of plant membrane lipids by multiple solid-phase extraction. J Chromatogr A 2001;922:347–54. https://doi.org/10.1016/S0021-9673(01)00888-3.
[230] Schulte E. Trennung von Triglyceriden nach Kettenlänge und Sättigungsgrad durch HPLC. Fette Seifen Anstrichmittel 1981;83:289–91. https://doi.org/10.1002/lipi.19810830802.
[231] Privett OS, Blank ML. A method for the structural analysis of triglycerides and lecithins. J Am Oil Chem Soc 1963;40:70–5. https://doi.org/10.1007/BF02654747.
[232] Rey F, Lopes D, Maciel E, Monteiro J, Skjermo J, Funderud J, et al. Polar lipid profile of *Saccharina latissima*, a functional food from the sea. Algal Res 2019;39:101473. https://doi.org/10.1016/j.algal.2019.101473.
[233] Rey F, da Costa E, Campos AM, Cartaxana P, Maciel E, Domingues P, et al. Kleptoplasty does not promote major shifts in the lipidome of macroalgal chloroplasts sequestered by the sacoglossan sea slug *Elysia viridis*. Sci Rep 2017;7:11502. https://doi.org/10.1038/s41598-017-12008-z.
[234] Fried B, Sherma J. Thin-layer chromatography. New York: Marcel Dekker; 1999.
[235] Fuchs B, Süß R, Teuber K, Eibisch M, Schiller J. Lipid analysis by thin-layer chromatography—A review of the current state. J Chromatogr A 2011;1218:2754–74. https://doi.org/10.1016/j.chroma.2010.11.066.
[236] Dobson G, Christie WW, Nikolova-Damyanova B. Silver ion chromatography of lipids and fatty acids. J Chromatogr B Biomed Appl 1995;671:197–222. https://doi.org/10.1016/0378-4347(95)00157-e.
[237] Sherma J, Bennett S. Comparison of reagents for lipid and phospholipid detection and densitometric quantitation on silica gel and C18 reversed phase thin layers. J Liq Chromatogr 1983;6:1193–211. https://doi.org/10.1080/01483918308079991.
[238] Bouvier-Navé P, Berna A, Noiriel A, Compagnon V, Carlsson AS, Banas A, et al. Involvement of the phospholipid sterol Acyltransferase1 in plant sterol homeostasis and leaf senescence. Plant Physiol 2010;152:107–19. https://doi.org/10.1104/pp.109.145672.
[239] Vieler A, Wilhelm C, Goss R, Süss R, Schiller J. The lipid composition of the unicellular green alga *Chlamydomonas reinhardtii* and the diatom *Cyclotella meneghiniana* investigated by MALDI-TOF MS and TLC. Chem Phys Lipids 2007;150:143–55. https://doi.org/10.1016/j.chemphyslip.2007.06.224.
[240] Waksmundzka-Hajnos M, Sherma J, Kowalska T, editors. Thin layer chromatography in Phytochemistry. Boca Raton: CRC Press; 2008. https://doi.org/10.1201/9781420046786.
[241] Anyakudo F, Adams E, Van Schepdael A. Thin-layer chromatography–flame ionization detection. Chromatographia 2020:83. https://doi.org/10.1007/s10337-019-03849-z.
[242] Byreddy AR, Gupta A, Barrow CJ, Puri M. A quick colorimetric method for total lipid quantification in microalgae. J Microbiol Methods 2016;125:28–32. https://doi.org/10.1016/j.mimet.2016.04.002.
[243] Cheng Y-S, Zheng Y, VanderGheynst JS. Rapid quantitative analysis of lipids using a colorimetric method in a microplate format. Lipids 2011;46:95–103. https://doi.org/10.1007/s11745-010-3494-0.
[244] Knight JA, Anderson S, Rawle JM. Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. Clin Chem 1972;18:199–202.
[245] Bartlett EM, Lewis DH. Spectrophotometric determination of phosphate esters in the presence and absence of orthophosphate. Anal Biochem 1970;36:159–67. https://doi.org/10.1016/0003-2697(70)90343-x.
[246] Chapman GW. A conversion factor to determine phospholipid content in soybean and sunflower crude oils. J Am Oil Chem Soc 1980;57:299–302. https://doi.org/10.1007/BF02662211.
[247] Ames BN. Assay of inorganic phosphate, total phosphate and phosphatases. Methods Enzymol 1966;8:115–8. https://doi.org/10.1016/0076-6879(66)08014-5. Academic Press.
[248] Watanabe K, Arao Y. A new solvent system for the separation of neutral glycosphingolipids. J Lipid Res 1981;22:1020–4. https://doi.org/10.1016/S0022-2275(20)37340-5.
[249] Bell BM, Daniels DGH, Fearn T, Stewart BA. Lipid compositions, baking qualities and other characteristics of wheat varieties grown in the U.K. J Cereal Sci 1987;5:277–86. https://doi.org/10.1016/S0733-5210(87)80028-0.
[250] Brands M, Gutbrod P, Dörmann P. Lipid analysis by gas chromatography and gas chromatography–mass spectrometryspectrometry. In: Bartels D, Dörmann P, editors. Plant Lipids: Methods and protocols. New York, NY: Springer US; 2021. p. 43–57. https://doi.org/10.1007/978-1-0716-1362-7_4.
[251] Griffiths MJ, van Hille RP, Harrison STL. Selection of direct transesterification as the preferred method for assay of fatty acid content of microalgae. Lipids 2010;45:1053–60. https://doi.org/10.1007/s11745-010-3468-2.
[252] Berdeaux O, Juaneda P, Martine L, Cabaret S, Bretillon L, Acar N. Identification and quantification of phosphatidylcholines containing very-long-chain polyunsaturated fatty acid in bovine and human retina using liquid chromatography/tandem mass spectrometry. J Chromatogr A 2010;1217:7738–48. https://doi.org/10.1016/j.chroma.2010.10.039.
[253] Christie WW, Han X. Chapter 8 - gas chromatographic analysis of fatty acid derivatives. In: Christie WW, Han X, editors. Lipid analysis. 4th ed. Woodhead Publishing; 2012. p. 159–80. https://doi.org/10.1533/9780857097866.159.
[254] Bouvier-Navé P, Benveniste P, Oelkers P, Sturley SL, Schaller H. Expression in yeast and tobacco of plant cDNAs encoding acyl CoA:diacylglycerol acyltransferase. Eur J Biochem 2000;267:85–96. https://doi.org/10.1046/j.1432-1327.2000.00961.x.
[255] Winkler JK, Rennick KA, Eller FJ, Vaughn SF. Phytosterol and tocopherol components in extracts of corn Distiller's dried grain. J Agric Food Chem 2007;55:6482–6. https://doi.org/10.1021/jf070594q.
[256] Cacas J-L, Melser S, Domergue F, Joubès J, Bourdenx B, Schmitter J-M, et al. Rapid nanoscale quantitative analysis of plant sphingolipid long-chain bases by GC-MS. Anal Bioanal Chem 2012;403:2745–55. https://doi.org/10.1007/s00216-012-6060-1.
[257] Pascal S, Bernard A, Deslous P, Gronnier J, Fournier-Goss A, Domergue F, et al. Arabidopsis CER1-LIKE1 functions in a cuticular very-long-chain alkane-forming Complex1. Plant Physiol 2019;179:415–32. https://doi.org/10.1104/pp.18.01075.
[258] Dodds ED, McCoy MR, Rea LD, Kennish JM. Gas chromatographic quantification of fatty acid methyl esters: flame ionization detection vs. Electron impact mass spectrometry. Lipids 2005;40:419–28. https://doi.org/10.1007/s11745-006-1399-8.
[259] Stefanoudaki E, Kotsifaki F, Koutsaftakis A. Classification of virgin olive oils of the two major cretan cultivars based on their fatty acid composition. J Am Oil Chem Soc 1999;76:623–6. https://doi.org/10.1007/s11746-999-0013-7.
[260] Kehelpannala C, Rupasinghe T, Pasha A, Esteban E, Hennessy T, Bradley D, et al. An Arabidopsis lipid map reveals differences between tissues and dynamic changes throughout development. Plant J 2021;107:287–302. https://doi.org/10.1111/tpj.15278.
[261] Alves E, Domingues M, Domingues P. Polar lipids from olives and olive oil: A review on their identification, significance and potential biotechnological applications. Foods 2018;7:1–14. https://doi.org/10.3390/foods7070109.
[262] Nikolaichuk H, Przykaza K, Kozub A, Montowska M, Wójcicka G, Tomaszewska-Gras J, et al. Shotgun lipidomic analysis for differentiation of niche cold pressed oils. Molecules 2022;27:1848. https://doi.org/10.3390/molecules27061848.
[263] Liu G-J, Xiao G-H, Liu N-J, Liu D, Chen P-S, Qin Y-M, et al. Targeted lipidomics studies reveal that linolenic acid promotes cotton fiber elongation by activating phosphatidylinositol and phosphatidylinositol monophosphate biosynthesis. Mol Plant 2015;8:911–21. https://doi.org/10.1016/j.molp.2015.02.010.
[264] Vu HS, Shiva S, Roth MR, Tamura P, Zheng L, Li M, et al. Lipid changes after leaf wounding in *Arabidopsis thaliana*: expanded lipidomic data form the basis for lipid co-occurrence analysis. Plant J 2014;80:728–43. https://doi.org/10.1111/tpj.12659.
[265] Holguin FO, Schaub T. Characterization of microalgal lipid feedstock by direct-infusion FT-ICR mass spectrometry. Algal Res 2013;2:43–50. https://doi.org/10.1016/j.algal.2012.11.005.
[266] Alves E, Melo T, Barros MP, Domingues MRM, Domingues P. Lipidomic profiling of the olive (*Olea europaea* L.) fruit towards its valorisation as a functional food: in-depth identification of triacylglycerols and polar lipids in Portuguese olives. Molecules 2019;24:2555. https://doi.org/10.3390/molecules24142555.
[267] Maciel E, Lillebø A, Domingues P, da Costa E, Calado R, Domingues MRM. Polar lipidome profiling of *Salicornia ramosissima* and *Halimione portulacoides* and the relevance of lipidomics for the valorization of halophytes. Phytochemistry 2018;153:94–101. https://doi.org/10.1016/j.phytochem.2018.05.015.
[268] Lopes D, Melo T, Meneses J, Abreu MH, Pereira R, Domingues P, et al. A new look for the red macroalga *Palmaria palmata*: A seafood with polar lipids rich in EPA and with antioxidant properties. Mar Drugs 2019;17:533. https://doi.org/10.3390/md17090533.
[269] Santos F, Monteiro JP, Duarte D, Melo T, Lopes D, da Costa E, et al. Unraveling the Lipidome and antioxidant activity of native *Bifurcaria bifurcata* and invasive *Sargassum muticum* seaweeds: A lipid perspective on how systemic intrusion may present an opportunity. Antioxidants 2020;9:642. https://doi.org/10.3390/antiox9070642.
[270] Aveiro SS, Melo T, Figueiredo A, Domingues P, Pereira H, Maia IB, et al. The polar lipidome of cultured *Emiliania huxleyi*: A source of bioactive lipids with relevance for biotechnological applications. Biomolecules 2020;10:1434. https://doi.org/10.3390/biom10101434.
[271] Couto D, Melo T, Conde TA, Moreira ASP, Ferreira P, Costa M, et al. Food grade extraction of *Chlorella vulgaris* polar lipids: A comparative lipidomic study. Food Chem 2022;375:131685. https://doi.org/10.1016/j.foodchem.2021.131685.
[272] Melo T, Figueiredo ARP, da Costa E, Couto D, Silva J, Domingues MR, et al. Ethanol extraction of polar lipids from Nannochloropsis oceanica for food, feed, and biotechnology applications evaluated using lipidomic approaches. Mar Drugs 2021;19:593. https://doi.org/10.3390/md19110593.
[273] Cajka T, Fiehn O. Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry. TrAC Trends Anal Chem 2014;61:192–206. https://doi.org/10.1016/j.trac.2014.04.017.

[274] Alves E, Melo T, Barros MP, Domingues MRM, Domingues P. _Lipidomic profiling of the olive (*Olea europaea* L.) fruit towards its valorisation as a functional food: in-depth identification of triacylglycerols and polar lipids in Portuguese olives. Molecules 2019;24:1–22. https://doi.org/10.3390/molecules24142555.
[275] da Costa E, Melo T, Reis M, Domingues P, Calado R, Abreu MH, et al. Polar lipids composition, antioxidant and anti-inflammatory activities of the Atlantic red seaweed Grateloupia turuturu. Mar Drugs 2021;19:414. https://doi.org/10.3390/md19080414.
[276] Navarro-Reig M, Jaumot J, Tauler R. An untargeted lipidomic strategy combining comprehensive two-dimensional liquid chromatography and chemometric analysis. J Chromatogr A 2018;1568:80–90. https://doi.org/10.1016/j.chroma.2018.07.017.
[277] Pham TH, Zaeem M, Fillier TA, Nadeem M, Vidal NP, Manful C, et al. Targeting modified lipids during routine lipidomics analysis using HILIC and C30 reverse phase liquid chromatography coupled to mass spectrometry. Sci Rep 2019;9:5048. https://doi.org/10.1038/s41598-019-41556-9.
[278] Burgos A, Szymanski J, Seiwert B, Degenkolbe T, Hannah MA, Giavalisco P, et al. Analysis of short-term changes in the *Arabidopsis thaliana* glycerolipidome in response to temperature and light. Plant J 2011;66:656–68. https://doi.org/10.1111/j.1365-313X.2011.04531.x.
[279] Hummel J, Segu S, Li Y, Irgang S, Jueppner J, Giavalisco P. Ultra performance liquid chromatography and high resolution mass spectrometry for the analysis of plant lipids. Front Plant Sci 2011;2:54. https://doi.org/10.3389/fpls.2011.00054.
[280] Liu M-Y, Burgos A, Ma L, Zhang Q, Tang D, Ruan J. Lipidomics analysis unravels the effect of nitrogen fertilization on lipid metabolism in tea plant (*Camellia sinensis* L.). BMC Plant Biol 2017;17:165. https://doi.org/10.1186/s12870-017-1111-6.
[281] Giavalisco P, Li Y, Matthes A, Eckhardt A, Hubberten H-M, Hesse H, et al. Elemental formula annotation of polar and lipophilic metabolites using 13C, 15N and 34S isotope labelling, in combination with high-resolution mass spectrometry. Plant J 2011;68:364–76. https://doi.org/10.1111/j.1365-313X.2011.04682.x.
[282] Lapidot-Cohen T, Rosental L, Brotman Y. Liquid chromatography-mass spectrometry (LC-MS)-based analysis for lipophilic compound profiling in plants. Curr Protoc Plant Biol 2020;5:e20109. https://doi.org/10.1002/cppb.20109.
[283] Lu S, Liu H, Jin C, Li Q, Guo L. An efficient and comprehensive plant glycerolipids analysis approach based on high-performance liquid chromatography-quadrupole time-of-flight mass spectrometer. Plant Direct 2019;3:e00183. https://doi.org/10.1002/pld3.183.
[284] Chollet C, Boutet-Mercey S, Laboureur L, Rincon C, Méjean M, Jouhet J, et al. Supercritical fluid chromatography coupled to mass spectrometry for lipidomics. J Mass Spectrom 2019;54:791–801. https://doi.org/10.1002/jms.4445.
[285] Buré C, Cacas J-L, Mongrand S, Schmitter J-M. Characterization of glycosyl inositol phosphoryl ceramides from plants and fungi by mass spectrometry. Anal Bioanal Chem 2014;406:995–1010. https://doi.org/10.1007/s00216-013-7130-8.
[286] Khoury S, Canlet C, Lacroix MZ, Berdeaux O, Jouhet J, Bertrand-Michel J. Quantification of Lipids: Model, reality, and compromise. Biomolecules 2018;8:174. https://doi.org/10.3390/biom8040174.
[287] Wang M, Wang C, Han X. Selection of internal standards for accurate quantification of complex lipid species in biological extracts by electrospray ionization mass spectrometry—what, how and why? Mass Spectrom Rev 2017;36:693–714. https://doi.org/10.1002/mas.21492.
[288] Welti R, Li W, Li M, Sang Y, Biesiada H, Zhou H-E, et al. Profiling membrane Lipids in plant stress responses: Role of phospholipase dα in freezing-induced lipid changes in arabidopsis*. J Biol Chem 2002;277:31994–2002. https://doi.org/10.1074/jbc.M205375200.
[289] Heyman HM, Dubery IA. The potential of mass spectrometry imaging in plant metabolomics: a review. Phytochem Rev 2016;15:297–316. https://doi.org/10.1007/s11101-015-9416-2.
[290] Boughton BA, Thinagaran D, Sarabia D, Bacic A, Roessner U. Mass spectrometry imaging for plant biology: a review. Phytochem Rev 2016;15:445–88. https://doi.org/10.1007/s11101-015-9440-2.
[291] Pathmasiri KC, Nguyen TTA, Khamidova N, Cologna SM. Chapter ten - mass spectrometry-based lipid analysis and imaging. In: Model MA, Levitan I, editors. Current topics in membranes. vol. 88. Academic Press; 2021. p. 315–57. https://doi.org/10.1016/bs.ctm.2021.10.005.
[292] Dueñas ME, Klein AT, Alexander LE, Yandeau-Nelson MD, Nikolau BJ, Lee YJ. High spatial resolution mass spectrometry imaging reveals the genetically programmed, developmental modification of the distribution of thylakoid membrane lipids among individual cells of maize leaf. Plant J 2017;89:825–38. https://doi.org/10.1111/tpj.13422.
[293] Woodfield HK, Sturtevant D, Borisjuk L, Munz E, Guschina IA, Chapman K, et al. Spatial and temporal mapping of key lipid species in *Brassica napus* seeds. Plant Physiol 2017;173:1998–2009. https://doi.org/10.1104/pp.16.01705.
[294] Dong Y, Li B, Malitsky S, Rogachev I, Aharoni A, Kaftan F, et al. Sample preparation for mass spectrometry imaging of plant tissues: A review. Front Plant Sci 2016:7.
[295] Horn PJ, Chapman KD. Lipidomics *in situ*: insights into plant lipid metabolism from high resolution spatial maps of metabolites. Prog Lipid Res 2014;54:32–52. https://doi.org/10.1016/j.plipres.2014.01.003.
[296] Touboul D, Laprévote O, Brunelle A. Micrometric molecular histology of lipids by mass spectrometry imaging. Curr Opin Chem Biol 2011;15:725–32. https://doi.org/10.1016/j.cbpa.2011.04.017.
[297] Caprioli RM, Farmer TB, Gile J. Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS. Anal Chem 1997;69:4751–60. https://doi.org/10.1021/ac970888i.
[298] Laiko VV, Baldwin MA, Burlingame AL. Atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry. Anal Chem 2000;72:652–7. https://doi.org/10.1021/ac990998k.
[299] Zemski Berry KA, Hankin JA, Barkley RM, Spraggins JM, Caprioli RM, Murphy RC. MALDI imaging of lipid biochemistry in tissues by mass spectrometry. Chem Rev 2011;111:6491–512. https://doi.org/10.1021/cr200280p.
[300] Korte AR, Yandeau-Nelson MD, Nikolau BJ, Lee YJ. Subcellular-level resolution MALDI-MS imaging of maize leaf metabolites by MALDI-linear ion trap-Orbitrap mass spectrometer. Anal Bioanal Chem 2015;407:2301–9. https://doi.org/10.1007/s00216-015-8460-5.
[301] Engel KM, Prabutzki P, Leopold J, Nimptsch A, Lemmnitzer K, Vos DRN, et al. A new update of MALDI-TOF mass spectrometry in lipid research. Prog Lipid Res 2022;86:101145. https://doi.org/10.1016/j.plipres.2021.101145.
[302] Takáts Z, Wiseman JM, Gologan B, Cooks RG. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. Science 2004;306:471–3. https://doi.org/10.1126/science.1104404.
[303] Müller T, Oradu S, Ifa DR, Cooks RG, Kräutler B. Direct plant tissue analysis and imprint imaging by desorption electrospray ionization mass spectrometry. Anal Chem 2011;83:5754–61. https://doi.org/10.1021/ac201123t.
[304] Manicke NE, Wiseman JM, Ifa DR, Cooks RG. Desorption electrospray ionization (DESI) mass spectrometry and tandem mass spectrometry (MS/MS) of phospholipids and sphingolipids: ionization, adduct formation, and fragmentation. J Am Soc Mass Spectrom 2008;19:531–43. https://doi.org/10.1016/j.jasms.2007.12.003.
[305] Unsihuay D, Qiu J, Swaroop S, Nagornov KO, Kozhinov AN, Tsybin YO, et al. Imaging of triglycerides in tissues using nanospray desorption electrospray ionization (Nano-DESI) mass spectrometry. Int J Mass Spectrom 2020;448:116269. https://doi.org/10.1016/j.ijms.2019.116269.
[306] Liu B, Meng X, Li K, Guo J, Cai Z. Visualization of lipids in cottonseeds by matrix-assisted laser desorption/ionization mass spectrometry imaging. Talanta 2021;221:121614. https://doi.org/10.1016/j.talanta.2020.121614.
[307] Bien T, Hambleton EA, Dreisewerd K, Soltwisch J. Molecular insights into symbiosis—mapping sterols in a marine flatworm-algae-system using high spatial resolution MALDI-2-MS imaging with ion mobility separation. Anal Bioanal Chem 2021;413:2767–77. https://doi.org/10.1007/s00216-020-03070-0.
[308] Passarelli MK, Pirkl A, Moellers R, Grinfeld D, Kollmer F, Havelund R, et al. The 3D OrbiSIMS—label-free metabolic imaging with subcellular lateral resolution and high mass-resolving power. Nat Methods 2017;14:1175–83. https://doi.org/10.1038/nmeth.4504.
[309] Largeau C, Casadevall E, Berkaloff C, Dhamelincourt P. Sites of accumulation and composition of hydrocarbons in *Botryococcus braunii*. Phytochemistry 1980;19:1043–51. https://doi.org/10.1016/0031-9422(80)83054-8.
[310] Samek O, Jonáš A, Pilát Z, Zemánek P, Nedbal L, Tříska J, et al. Raman microspectroscopy of individual algal cells: sensing unsaturation of storage lipids in vivo. Sensors 2010;10:8635–51. https://doi.org/10.3390/s100908635.
[311] Huang YY, Beal CM, Cai WW, Ruoff RS, Terentjev EM. Micro-Raman spectroscopy of algae: composition analysis and fluorescence background behavior. Biotechnol Bioeng 2010;105:889–98. https://doi.org/10.1002/bit.22617.
[312] Weiss TL, Chun HJ, Okada S, Vitha S, Holzenburg A, Laane J, et al. Raman spectroscopy analysis of botryococcene hydrocarbons from the green microalga *Botryococcus braunii**. J Biol Chem 2010;285:32458–66. https://doi.org/10.1074/jbc.M110.157230.
[313] Lee T-H, Chang J-S, Wang H-Y. Rapid and in vivo quantification of cellular lipids in *Chlorella vulgaris* using near-infrared Raman spectrometry. Anal Chem 2013;85:2155–60. https://doi.org/10.1021/ac3028118.
[314] Wang T, Ji Y, Wang Y, Jia J, Li J, Huang S, et al. Quantitative dynamics of triacylglycerol accumulation in microalgae populations at single-cell resolution revealed by Raman microspectroscopy. Biotechnol Biofuels 2014;7:58. https://doi.org/10.1186/1754-6834-7-58.
[315] Chiu L, Ho S-H, Shimada R, Ren N-Q, Ozawa T. Rapid in vivo lipid/carbohydrate quantification of single microalgal cell by Raman spectral imaging to reveal salinity-induced starch-to-lipid shift. Biotechnol Biofuels 2017;10:9. https://doi.org/10.1186/s13068-016-0691-y.
[316] Kim HS, Waqued SC, Nodurft DT, Devarenne TP, Yakovlev VV, Han A. Raman spectroscopy compatible PDMS droplet microfluidic culture and analysis platform towards on-chip lipidomics. Analyst 2017;142:1054–60. https://doi.org/10.1039/C6AN02221A.
[317] Hosokawa M, Ando M, Mukai S, Osada K, Yoshino T, Hamaguchi H, et al. In vivo live cell imaging for the quantitative monitoring of lipids by using Raman microspectroscopy. Anal Chem 2014;86:8224–30. https://doi.org/10.1021/ac501591d.
[318] He Y, Zhang P, Huang S, Wang T, Ji Y, Xu J. Label-free, simultaneous quantification of starch, protein and triacylglycerol in single microalgal cells. Biotechnol Biofuels 2017;10:275. https://doi.org/10.1186/s13068-017-0967-x.
[319] Li X, Sha J, Chu B, Wei Y, Huang W, Zhou H, et al. Quantitative visualization of intracellular lipids concentration in a microalgae cell based on Raman micro-spectroscopy coupled with chemometrics. Sensors Actuators B Chem 2019;292:7–15. https://doi.org/10.1016/j.snb.2019.04.048.
[320] Ji Y, He Y, Cui Y, Wang T, Wang Y, Li Y, et al. Raman spectroscopy provides a rapid, non-invasive method for quantitation of starch in live, unicellular microalgae. Biotechnol J 2014;9:1512–8. https://doi.org/10.1002/biot.201400165.

[321] Moudříková Š, Sadowsky A, Metzger S, Nedbal L, Mettler-Altmann T, Mojzeš P. Quantification of polyphosphate in microalgae by Raman microscopy and by a reference enzymatic assay. Anal Chem 2017;89:12006–13. https://doi.org/10.1021/acs.analchem.7b02393.

[322] Moudříková Š, Mojzeš P, Zachleder V, Pfaff C, Behrendt D, Nedbal L. Raman and fluorescence microscopy sensing energy-transducing and energy-storing structures in microalgae. Algal Res 2016;16:224–32. https://doi.org/10.1016/j.algal.2016.03.016.

[323] Mojzeš P, Gao L, Ismagulova T, Pilátová J, Moudříková Š, Gorelova O, et al. Guanine, a high-capacity and rapid-turnover nitrogen reserve in microalgal cells. Proc Natl Acad Sci 2020;117:32722–30. https://doi.org/10.1073/pnas.2005460117.

[324] Moudříková Š, Ivanov IN, Vítová M, Nedbal L, Zachleder V, Mojzeš P, et al. Comparing biochemical and Raman microscopy analyses of starch, Lipids, polyphosphate, and guanine pools during the cell cycle of Desmodesmus quadricauda. Cells 2021;10:62. https://doi.org/10.3390/cells10010062.

[325] Heidari Baladehi M, Hekmatara M, He Y, Bhaskar Y, Wang Z, Liu L, et al. Culture-free identification and metabolic profiling of microalgal single cells via ensemble learning of Ramanomes. Anal Chem 2021;93:8872–80. https://doi.org/10.1021/acs.analchem.1c01015.

[326] Cavonius L, Fink H, Kiskis J, Albers E, Undeland I, Enejder A. Imaging of Lipids in microalgae with coherent anti-stokes Raman scattering microscopy. Plant Physiol 2015;167:603–16. https://doi.org/10.1104/pp.114.252197.

[327] Jaeger D, Pilger C, Hachmeister H, Oberländer E, Wördenweber R, Wichmann J, et al. Label-free in vivo analysis of intracellular lipid droplets in the oleaginous microalga Monoraphidium neglectum by coherent Raman scattering microscopy. Sci Rep 2016;6:35340. https://doi.org/10.1038/srep35340.

[328] Wu H, Volponi JV, Oliver AE, Parikh AN, Simmons BA, Singh S. In vivo lipidomics using single-cell Raman spectroscopy. Proc Natl Acad Sci 2011;108:3809–14. https://doi.org/10.1073/pnas.1009043108.

[329] Meksiarun P, Spegazzini N, Matsui H, Nakajima K, Matsuda Y, Sato H. In vivo study of lipid accumulation in the microalgae marine diatom *Thalassiosira pseudonana* using Raman spectroscopy. Appl Spectrosc 2015;69:45–51. https://doi.org/10.1366/14-07598.

[330] Sharma SK, Nelson DR, Abdrabu R, Khraiwesh B, Jijakli K, Arnoux M, et al. An integrative Raman microscopy-based workflow for rapid in situ analysis of microalgal lipid bodies. Biotechnol Biofuels 2015;8:164. https://doi.org/10.1186/s13068-015-0349-1.

[331] Li K, Cheng J, Ye Q, He Y, Zhou J, Cen K. In vivo kinetics of lipids and astaxanthin evolution in *Haematococcus pluvialis* mutant under 15% CO2 using Raman microspectroscopy. Bioresour Technol 2017;244:1439–44. https://doi.org/10.1016/j.biortech.2017.04.116.

[332] Pilát Z, Bernatová S, Ježek J, Šerý M, Samek O, Zemánek P, et al. Raman microspectroscopy of algal lipid bodies: β-carotene quantification. J Appl Phycol 2012;24:541–6. https://doi.org/10.1007/s10811-011-9754-4.

[333] Fu D, Lu F-K, Zhang X, Freudiger C, Pernik DR, Holtom G, et al. Quantitative chemical imaging with multiplex stimulated Raman scattering microscopy. J Am Chem Soc 2012;134:3623–6. https://doi.org/10.1021/ja210081h.

[334] Davis RW, Jones HDT, Collins AM, Ricken JB, Sinclair MB, Timlin JA, et al. Label-free measurement of algal triacylglyceride production using fluorescence hyperspectral imaging. Algal Res 2014;5:181–9. https://doi.org/10.1016/j.algal.2013.11.010.

[335] Laurens LML, Wolfrum EJ. Feasibility of spectroscopic characterization of algal Lipids: Chemometric correlation of NIR and FTIR spectra with exogenous Lipids in algal biomass. Bioenergy Res 2011;4:22–35. https://doi.org/10.1007/s12155-010-9098-y.

[336] Dean AP, Sigee DC, Estrada B, Pittman JK. Using FTIR spectroscopy for rapid determination of lipid accumulation in response to nitrogen limitation in freshwater microalgae. Bioresour Technol 2010;101:4499–507. https://doi.org/10.1016/j.biortech.2010.01.065.

[337] Jebsen C, Norici A, Wagner H, Palmucci M, Giordano M, Wilhelm C. FTIR spectra of algal species can be used as physiological fingerprints to assess their actual growth potential. Physiol Plant 2012:146. https://doi.org/10.1111/j.1399-3054.2012.01636.x.

[338] Grace C Esther Elizabeth, Lakshmi P Kiruthika, Meenakshi S, Vaidyanathan S, Srisudha S, Mary M Briget. Biomolecular transitions and lipid accumulation in green microalgae monitored by FTIR and Raman analysis. Spectrochim Acta A Mol Biomol Spectrosc 2020;224:117382. https://doi.org/10.1016/j.saa.2019.117382.

[339] Grube M, Shvirksts K, Krafft C, Kokorevicha S, Zandberga E, Abols A, et al. Miniature diamond-anvil cells for FTIR-microspectroscopy of small quantities of biosamples. Analyst 2018;143:3595–9. https://doi.org/10.1039/C8AN00432C.

[340] Scarsini M, Thurotte A, Veidl B, Amiard F, Niepceron F, Badawi M, et al. Metabolite quantification by fourier transform infrared spectroscopy in diatoms: proof of concept on *Phaeodactylum tricornutum*. Front Plant Sci 2021:12.

[341] Naumann D, Meyers (ed RA, Naumann D. Infrared spectroscopy in n.d.

[342] Grube M, Bekers M, Upite D, Kaminska E. IR-spectroscopic studies of Zymomonas mobilis and Levan precipitate. Vib Spectrosc 2002;28:277–85. https://doi.org/10.1016/S0924-2031(02)00005-X.

[343] Agatonovic-Kustrin S, Ramenskaya G, Kustrin E, Ortakand DB, Morton DW. A new integrated HPTLC - ATR/FTIR approach in marine algae bioprofiling. J Pharm Biomed Anal 2020;189:113488. https://doi.org/10.1016/j.jpba.2020.113488.

[344] Carnovale G, Rosa F, Shapaval V, Dzurendova S, Kohler A, Wicklund T, et al. Starch rich *Chlorella vulgaris*: high-throughput screening and up-scale for tailored biomass production. Appl Sci 2021;11:9025. https://doi.org/10.3390/app11199025.

[345] Ferreira R, Lourenço S, Lopes A, Andrade C, Câmara JS, Castilho P, et al. Evaluation of fatty acids profile as a useful tool towards valorization of by-products of Agri-food industry. Foods 2021;10:2867. https://doi.org/10.3390/foods10112867.

[346] Berzins K, Muiznieks R, Baumanis MR, Strazdina I, Shvirksts K, Prikule S, et al. Kinetic and stoichiometric modeling-based analysis of docosahexaenoic acid (DHA) production potential by *Crypthecodinium cohnii* from glycerol, glucose and ethanol. Mar Drugs 2022;20:115. https://doi.org/10.3390/md20020115.

[347] Bisht B, Kumar V, Gururani P, Tomar MS, Nanda M, Vlaskin MS, et al. The potential of nuclear magnetic resonance (NMR) in metabolomics and lipidomics of microalgae- a review. Arch Biochem Biophys 2021;710:108987. https://doi.org/10.1016/j.abb.2021.108987.

[348] Nuzzo G, Gallo C, d'Ippolito G, Cutignano A, Sardo A, Fontana A. Composition and quantitation of microalgal Lipids by ERETIC 1H NMR method. Mar Drugs 2013;11:3742–53. https://doi.org/10.3390/md11103742.

[349] Sarpal AS, Teixeira CMLL, Silva PRM, da Costa Monteiro TV, da Silva JI, da Cunha VS, et al. NMR techniques for determination of lipid content in microalgal biomass and their use in monitoring the cultivation with biodiesel potential. Appl Microbiol Biotechnol 2016;100:2471–85. https://doi.org/10.1007/s00253-015-7140-x.

[350] Pollesello P, Toffanin R, Murano E, Rizzo R, Paoletti S, Kvam BJ. 1H- and13C-NMR spectroscopic studies of lipid extracts of the red alga Gracilaria longa. J Appl Phycol 1992;4:149–55. https://doi.org/10.1007/BF02442463.

[351] Vigli G, Philippidis A, Spyros A, Dais P. Classification of edible oils by employing 31P and 1H NMR spectroscopy in combination with multivariate statistical analysis. A proposal for the detection of seed oil adulteration in virgin olive oils. J Agric Food Chem 2003;51:5715–22. https://doi.org/10.1021/jf030100z.

[352] Dais P, Hatzakis E. Quality assessment and authentication of virgin olive oil by NMR spectroscopy: A critical review. Anal Chim Acta 2013;765:1–27. https://doi.org/10.1016/j.aca.2012.12.003.

[353] Hatzakis E, Koidis A, Boskou D, Dais P. Determination of phospholipids in olive oil by 31P NMR spectroscopy. J Agric Food Chem 2008;56:6232–40. https://doi.org/10.1021/jf800690t.

[354] Monakhova YB, Diehl BWK. Automated multicomponent phospholipid analysis using 31P NMR spectroscopy: Example of vegetable lecithin and krill oil. Anal Bioanal Chem 2018;410:7891–900. https://doi.org/10.1007/s00216-018-1408-9.

[355] Castro V, Dvinskikh SV, Widmalm G, Sandström D, Maliniak A. NMR studies of membranes composed of glycolipids and phospholipids. Biochim Biophys Acta Biomembr 2007;1768:2432–7. https://doi.org/10.1016/j.bbamem.2007.05.010.

[356] Samman S, Chow JWY, Foster MJ, Ahmad ZI, Phuyal JL, Petocz P. Fatty acid composition of edible oils derived from certified organic and conventional agricultural methods. Food Chem 2008;109:670–4. https://doi.org/10.1016/j.foodchem.2007.12.067.

[357] Xue Z, Li S, Yu W, Gao X, Zheng X, Yu Y, et al. Research advancement and commercialization of microalgae edible oil: a review. J Sci Food Agric 2021;101:5763–74. https://doi.org/10.1002/jsfa.11390.

[358] Xue Z, Yu Y, Yu W, Gao X, Zhang Y, Kou X. Development prospect and preparation technology of edible oil from microalgae. Front Mar Sci 2020:7.

[359] de Freitas CAS, de Sousa PHM, Soares DJ, da Silva JYG, Benjamin SR, Guedes MIF. Carnauba wax uses in food – A review. Food Chem 2019;291:38–48. https://doi.org/10.1016/j.foodchem.2019.03.133.

[360] Rios RV, Pessanha MDF, de Almeida PF, Viana CL, Lannes SC da S. Application of fats in some food products. Food. Sci Technol 2014;34:3–15. https://doi.org/10.1590/S0101-20612014000100001.

[361] Dj M. Emulsion design to improve the delivery of functional lipophilic components. Annu Rev Food Sci Technol 2010:1. https://doi.org/10.1146/annurev.food.080708.100722.

[362] Tan Y, McClements DJ. Improving the bioavailability of oil-soluble vitamins by optimizing food matrix effects: A review. Food Chem 2021;348:129148. https://doi.org/10.1016/j.foodchem.2021.129148.

[363] Bhat S, Maganja D, Huang L, Wu JHY, Marklund M. Influence of heating during cooking on trans fatty acid content of edible oils: A systematic review and Meta-analysis. Nutrients 2022;14:1489. https://doi.org/10.3390/nu14071489.

[364] Napier JA, Sayanova O. Nutritional enhancement in plants – green and greener. Curr Opin Biotechnol 2020;61:122–7. https://doi.org/10.1016/j.copbio.2019.12.010.

[365] Han L, Usher S, Sandgrind S, Hassall K, Sayanova O, Michaelson LV, et al. High level accumulation of EPA and DHA in field-grown transgenic Camelina – a multi-territory evaluation of TAG accumulation and heterogeneity. Plant Biotechnol J 2020;18:2280–91. https://doi.org/10.1111/pbi.13385.

[366] Betancor MB, Li K, Bucerzan VS, Sprague M, Sayanova O, Usher S, et al. Oil from transgenic Camelina sativa containing over 25% n-3 long-chain PUFA as the major lipid source in feed for Atlantic salmon (Salmo salar). Br J Nutr 2018;119:1378–92. https://doi.org/10.1017/S0007114518001125.

[367] Betancor MB, MacEwan A, Sprague M, Gong X, Montero D, Han L, et al. Oil from transgenic *Camelina sativa* as a source of EPA and DHA in feed for European sea bass (*Dicentrarchus labrax* L.). Aquaculture 2021;530:735759. https://doi.org/10.1016/j.aquaculture.2020.735759.

[368] Nawade B, Mishra GP, Radhakrishnan T, Dodia SM, Ahmad S, Kumar A, et al. High oleic peanut breeding: achievements, perspectives, and prospects. Trends Food Sci Technol 2018;78:107–19. https://doi.org/10.1016/j.tifs.2018.05.022.

[369] Aristizabal-Henao JJ, Stark KD. Macrolipidomic profiling of vegetable oils: The analysis of sunflower oils with different oleic acid content. In: Shulaev V, editor. Plant metabolic engineering: Methods and protocols. New York, NY: Springer US; 2022. p. 161–73. https://doi.org/10.1007/978-1-0716-1822-6_12.

[370] Matsuzawa Y, Higashi Y, Takano K, Takahashi M, Yamada Y, Okazaki Y, et al. Food Lipidomics for 155 agricultural plant products. J Agric Food Chem 2021;69:8981–90. https://doi.org/10.1021/acs.jafc.0c07356.
[371] Sturtevant D, Horn P, Kennedy C, Hinze L, Percy R, Chapman K. Lipid metabolites in seeds of diverse Gossypium accessions: Molecular identification of a high oleic mutant allele. Planta 2017;245:595–610. https://doi.org/10.1007/s00425-016-2630-3.
[372] Bakhytkyzy I, Hewelt-Belka W, Kot-Wasik A. A method for a comprehensive lipidomic analysis of flaxseed (*Linum usitatissimum*) with the use of LC–Q-TOF-MS and dispersive micro-solid-phase (μDSPE) extraction. Food Chem 2022;381:132290. https://doi.org/10.1016/j.foodchem.2022.132290.
[373] Richardson CE, Hennebelle M, Otoki Y, Zamora D, Yang J, Hammock BD, et al. Lipidomic analysis of oxidized fatty acids in plant and algae oils. J Agric Food Chem 2017;65:1941–51. https://doi.org/10.1021/acs.jafc.6b05559.
[374] Jadhav HB, Annapure U. Designer lipids -synthesis and application – A review. Trends Food Sci Technol 2021;116:884–902. https://doi.org/10.1016/j.tifs.2021.08.020.
[375] Sun J, Feng X, Lyu C, Zhou S, Liu Z. Effects of different processing methods on the lipid composition of hazelnut oil: A lipidomics analysis. Food Sci Human Wellness 2022;11:427–35. https://doi.org/10.1016/j.fshw.2021.11.024.
[376] Zhang D, Li X, Duan X, Sun H, Cao Y. Lipidomics reveals the changes in lipid profile of flaxseed oil affected by roasting. Food Chem 2021;364:130431. https://doi.org/10.1016/j.foodchem.2021.130431.
[377] Sun J, Hu P, Lyu C, Tian J, Meng X, Tan H, et al. Comprehensive lipidomics analysis of the lipids in hazelnut oil during storage. Food Chem 2022;378:132050. https://doi.org/10.1016/j.foodchem.2022.132050.
[378] Capriotti AL, Cerrato A, Aita SE, Montone CM, Piovesana S, Laganà A, et al. Degradation of the polar lipid and fatty acid molecular species in extra virgin olive oil during storage based on shotgun lipidomics. J Chromatogr A 2021;1639:461881. https://doi.org/10.1016/j.chroma.2021.461881.
[379] Righetti L, Rubert J, Galaverna G, Hurkova K, Dall'Asta C, Hajslova J, et al. A novel approach based on untargeted lipidomics reveals differences in the lipid pattern among durum and common wheat. Food Chem 2017:240. https://doi.org/10.1016/j.foodchem.2017.08.020.
[380] Shimazu T, Borjigin L, Katoh K, Roh S-G, Kitazawa H, Abe K, et al. Addition of Wakame seaweed (Undaria pinnatifida) stalk to animal feed enhances immune response and improves intestinal microflora in pigs. Anim Sci J 2019;90:1248–60. https://doi.org/10.1111/asj.13274.
[381] Yim D-G, Choi Y-S, Nam K-C. Sea tangle (Laminaria japonica) supplementation on meat quality of Korean native black goat. J Anim Sci Technol 2019;61:352–8. https://doi.org/10.5187/jast.2019.61.6.352.
[382] Diler I, A. Adem Tekinay, Derya Guroy, Betul Kut Guroy, Murat Soyuturk. Effects of *Ulva rigida* on the growth, feed intake and body composition of common carp, *Cyprinus carpio* L. J Biol Sci n.d.;7:305–8. doi:https://doi.org/10.3923/jbs.2007.305.308.
[383] Neijat M, Zacek P, Picklo MJ, House JD. Lipidomic characterization of omega-3 polyunsaturated fatty acids in phosphatidylcholine and phosphatidylethanolamine species of egg yolk lipid derived from hens fed flaxseed oil and marine algal biomass. Prostaglandins Leukot Essent Fat Acids 2020;161:102178. https://doi.org/10.1016/j.plefa.2020.102178.
[384] Conde TA, Zabetakis I, Tsoupras A, Medina I, Costa M, Silva J, et al. Microalgal lipid extracts have potential to modulate the inflammatory response: A critical review. IJMS 2021;22:1–15. https://doi.org/10.3390/ijms22189825.
[385] Sasso S, Pohnert G, Lohr M, Mittag M, Hertweck C. Microalgae in the postgenomic era: a blooming reservoir for new natural products. FEMS Microbiol Rev 2012;36:761–85. https://doi.org/10.1111/j.1574-6976.2011.00304.x.
[386] Gladyshev MI, Sushchik NN, Makhutova ON. Production of EPA and DHA in aquatic ecosystems and their transfer to the land. Prostaglandins Other Lipid Mediat 2013;107:117–26. https://doi.org/10.1016/j.prostaglandins.2013.03.002.
[387] Maeda N, Matsubara K, Yoshida H, Mizushina Y. Anti-cancer effect of spinach glycoglycerolipids as angiogenesis inhibitors based on the selective inhibition of DNA polymerase activity. Mini-Rev Med Chem 2011;11:32–8. https://doi.org/10.2174/138955711793564042.
[388] Abedin MR, Barua S. Isolation and purification of glycoglycerolipids to induce apoptosis in breast cancer cells. Sci Rep 2021;11:1298. https://doi.org/10.1038/s41598-020-80484-x.
[389] Christensen LP. Galactolipids as potential health promoting compounds in vegetable foods. Recent Pat Food Nutr Agric 2009;1:50–8. https://doi.org/10.2174/2212798410901010050.
[390] Wang R, Liu S, Liu T, Wu J, Zhang H, Sun Z, et al. Mass spectrometry-based serum lipidomics strategy to explore the mechanism of *Eleutherococcus senticosus* (Rupr. & maxim.) maxim. Leaves in the treatment of ischemic stroke. Food Funct 2021;12:4519–34. https://doi.org/10.1039/d0fo02845b.
[391] Shon JC, Kim WC, Ryu R, Wu Z, Seo J-S, Choi M-S, et al. Plasma Lipidomics reveals insights into anti-obesity effect of *Chrysanthemum morifolium* Ramat leaves and its constituent Luteolin in high-fat diet-induced Dyslipidemic mice. Nutrients 2020;12:E2973. https://doi.org/10.3390/nu12102973.
[392] Wu B, Li Y, Zhao W, Meng Z, Ji W, Wang C. Transcriptomic and Lipidomic analysis of Lipids in *Forsythia suspensa*. Front Genet 2021;12:758326. https://doi.org/10.3389/fgene.2021.758326.
[393] D'Urso G, Napolitano A, Cannavacciuolo C, Masullo M, Piacente S. Okra fruit: LC-ESI/LTQOrbitrap/MS/MSn based deep insight on polar lipids and specialized metabolites with evaluation of anti-oxidant and anti-hyperglycemic activity. Food Funct 2020;11:7856–65. https://doi.org/10.1039/D0FO00867B.
[394] Khozin-Goldberg I, Iskandarov U, Cohen Z. LC-PUFA from photosynthetic microalgae: occurrence, biosynthesis, and prospects in biotechnology. Appl Microbiol Biotechnol 2011;91:905–15. https://doi.org/10.1007/s00253-011-3441-x.
[395] Calder PC. Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. Biochim Biophys Acta 2015;1851:469–84. https://doi.org/10.1016/j.bbalip.2014.08.010.
[396] Yates CM, Calder PC, Ed Rainger G. Pharmacology and therapeutics of omega-3 polyunsaturated fatty acids in chronic inflammatory disease. Pharmacol Ther 2014;141:272–82. https://doi.org/10.1016/j.pharmthera.2013.10.010.
[397] Dasilva G, Medina I. Lipidomic methodologies for biomarkers of chronic inflammation in nutritional research: ω-3 and ω-6 lipid mediators. Free Radic Biol Med 2019:144. https://doi.org/10.1016/j.freeradbiomed.2019.03.017.
[398] Gabbs M, Leng S, Devassy JG, Monirujjaman M, Aukema HM. Advances in our understanding of Oxylipins derived from dietary PUFAs. Adv Nutr 2015;6:513–40. https://doi.org/10.3945/an.114.007732.
[399] Novichkova E, Chumin K, Eretz-Kdosha N, Boussiba S, Gopas J, Cohen G, et al. DGLA from the microalga Lobosphaera Incsa P127 modulates inflammatory response. In: Inhibits iNOS expression and alleviates NO secretion in RAW264.7 murine macrophages. vol. 12. Nutrients; 2020. E2892. https://doi.org/10.3390/nu12092892.
[400] Chiu C-Y, Gomolka B, Dierkes C, Huang NR, Schroeder M, Purschke M, et al. Omega-6 docosapentaenoic acid-derived resolvins and 17-hydroxydocosahexaenoic acid modulate macrophage function and alleviate experimental colitis. Inflamm Res 2012;61:967–76. https://doi.org/10.1007/s00011-012-0489-8.
[401] Han D, Jia J, Li J, Sommerfeld M, Xu J, Hu Q. Metabolic remodeling of membrane glycerolipids in the microalga nannochloropsis oceanica under nitrogen deprivation. Front Mar Sci 2017:4.
[402] Fan Y-Y, Chapkin RS. Importance of dietary γ-linolenic acid in human health and nutrition. J Nutr 1998;128:1411–4. https://doi.org/10.1093/jn/128.9.1411.
[403] Da Costa E, Silva J, Mendonça SH, Abreu MH, Domingues MR. Lipidomic approaches towards deciphering glycolipids from microalgae as a reservoir of bioactive lipids. Mar Drugs 2016;14:1–27. https://doi.org/10.3390/md14050101.
[404] Haslam R, Hamilton M, Economou C, Smith R, Hassall K, Napier J, et al. Overexpression of an endogenous type 2 diacylglycerol acyltransferase in the marine diatom *Phaeodactylum tricornutum* enhances lipid production and omega-3 long-chain polyunsaturated fatty acid content. Biotechnol Biofuels 2020:13. https://doi.org/10.1186/s13068-020-01726-8.
[405] Smith R, Jouhet J, Gandini C, Nekrasov V, Marechal E, Napier JA, et al. Plastidial acyl carrier protein Δ9-desaturase modulates eicosapentaenoic acid biosynthesis and triacylglycerol accumulation in *Phaeodactylum tricornutum*. Plant J 2021;106:1247–59. https://doi.org/10.1111/tpj.15231.
[406] Kokabi K, Gorelova O, Zorin B, Didi-Cohen S, Itkin M, Malitsky S, et al. Lipidome remodeling and autophagic respose in the arachidonic-acid-rich microalga Lobosphaera incisa under nitrogen and phosphorous deprivation. Front Plant Sci 2020:11.
[407] Di Costanzo F, Di Dato V, Ianora A, Romano G. Prostaglandins in marine organisms: A review. Mar Drugs 2019;17:428. https://doi.org/10.3390/md17070428.
[408] Vigor C, Oger C, Reversat G, Rocher A, Zhou B, Linares-Maurizi A, et al. Isoprostanoid profiling of marine microalgae. Biomolecules 2020;10:1073. https://doi.org/10.3390/biom10071073.
[409] Reifen R, Karlinsky A, Stark AH, Berkovich Z, Nyska A. α-Linolenic acid (ALA) is an anti-inflammatory agent in inflammatory bowel disease. J Nutr Biochem 2015;26:1632–40. https://doi.org/10.1016/j.jnutbio.2015.08.006.
[410] Ávila-Román J, Talero E, Alcaide A, De Los Reyes C, Zubía E, García-Mauriño S, et al. Preventive effect of the microalga Chlamydomonas debaryana on the acute phase of experimental colitis in rats. Br J Nutr 2014;112:1055–64. https://doi.org/10.1017/S0007114514001895.
[411] Ávila-Román J, Talero E, Rodríguez-Luna A, García-Mauriño S, Motilva V. Anti-inflammatory effects of an oxylipin-containing lyophilised biomass from a microalga in a murine recurrent colitis model. Br J Nutr 2016;116:2044–52. https://doi.org/10.1017/S0007114516004189.
[412] de los Reyes C, Ávila-Román J, Ortega MJ, de la Jara A, García-Mauriño S, Motilva V, et al. Oxylipins from the microalgae *Chlamydomonas debaryana* and *Nannochloropsis gaditana* and their activity as TNF-α inhibitors. Phytochemistry 2014;102:152–61. https://doi.org/10.1016/j.phytochem.2014.03.011.
[413] Di Dato V, Barbarinaldi R, Amato A, Di Costanzo F, Fontanarosa C, Perna A, et al. Variation in prostaglandin metabolism during growth of the diatom Thalassiosira rotula. Sci Rep 2020;10:5374. https://doi.org/10.1038/s41598-020-61967-3.
[414] Ávila-Román J, Talero E, de los Reyes C, García-Mauriño S, Motilva V. Microalgae-derived oxylipins decrease inflammatory mediators by regulating the subcellular location of NFκB and PPAR-γ. Pharmacol Res 2018;128:220–30. https://doi.org/10.1016/j.phrs.2017.10.009.
[415] Desbois AP, Lebl T, Yan L, Smith VJ. Isolation and structural characterisation of two antibacterial free fatty acids from the marine diatom, *Phaeodactylum tricornutum*. Appl Microbiol Biotechnol 2008;81:755–64. https://doi.org/10.1007/s00253-008-1714-9.
[416] Desbois AP, Mearns-Spragg A, Smith VJ. A fatty acid from the diatom Phaeodactylum tricornutum is antibacterial against diverse Bacteria including multi-resistant Staphylococcus aureus (MRSA). Mar Biotechnol 2009;11:45–52. https://doi.org/10.1007/s10126-008-9118-5.
[417] Alves E, Dias M, Lopes D, Almeida A, Domingues M do R, Rey F. Antimicrobial lipids from plants and marine organisms: an overview of the current state-of-the-

art and future prospects. Antibiotics 2020;9:441. https://doi.org/10.3390/antibiotics9080441.
[418] Davoodbasha M, Edachery B, Nooruddin T, Lee S-Y, Kim J-W. An evidence of C16 fatty acid methyl esters extracted from microalga for effective antimicrobial and antioxidant property. Microb Pathog 2018;115:233–8. https://doi.org/10.1016/j.micpath.2017.12.049.
[419] Abubakar MN, Majinda RRT. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and Pterocarpus angolensis (DC). Medicines 2016;3:3. https://doi.org/10.3390/medicines3010003.
[420] Salvador MJ, Ferreira EO, Pral EMF, Alfieri SC, Albuquerque S, Ito IY, et al. Bioactivity of crude extracts and some constituents of *Blutaparon portulacoides* (Amaranthaceae). Phytomedicine 2002;9:566–71. https://doi.org/10.1078/09447110260573227.
[421] Bharitkar YP, Bathini S, Ojha D, Ghosh S, Mukherjee H, Kuotsu K, et al. Antibacterial and antiviral evaluation of sulfonoquinovosyldiacylglyceride: a glycolipid isolated from *Azadirachta indica* leaves. Lett Appl Microbiol 2014;58:184–9. https://doi.org/10.1111/lam.12174.
[422] Wang H-MD, Chen C-C, Huynh P, Chang J-S. Exploring the potential of using algae in cosmetics. Bioresour Technol 2015;184:355–62. https://doi.org/10.1016/j.biortech.2014.12.001.
[423] Hughes K, Ho R, Butaud J-F, Filaire E, Ranouille E, Berthon J-Y, et al. A selection of eleven plants used as traditional Polynesian cosmetics and their development potential as anti-aging ingredients, hair growth promoters and whitening products. J Ethnopharmacol 2019;245:112159. https://doi.org/10.1016/j.jep.2019.112159.
[424] Colussi G, Catena C, Novello M, Bertin N, Sechi LA. Impact of omega-3 polyunsaturated fatty acids on vascular function and blood pressure: relevance for cardiovascular outcomes. Nutr Metab Cardiovasc Dis 2017;27:191–200. https://doi.org/10.1016/j.numecd.2016.07.011.
[425] Kubicki S, Bollinger A, Katzke N, Jaeger K-E, Loeschcke A, Thies S. Marine biosurfactants: biosynthesis, structural diversity and biotechnological applications. Mar Drugs 2019;17:408. https://doi.org/10.3390/md17070408.
[426] Velasquez-Orta SB, Lee JGM, Harvey AP. Evaluation of FAME production from wet marine and freshwater microalgae by *in situ* transesterification. Biochem Eng J 2013;76:83–9. https://doi.org/10.1016/j.bej.2013.04.003.
[427] De Luca M, Pappalardo I, Limongi AR, Viviano E, Radice RP, Todisco S, et al. Lipids from microalgae for cosmetic applications. Cosmetics 2021;8:52. https://doi.org/10.3390/cosmetics8020052.
[428] Zerres S, Stahl W. Carotenoids in human skin. Biochim Biophys Acta (BBA) - Mol Cell Biol Lipids 2020;1865:158588. https://doi.org/10.1016/j.bbalip.2019.158588.
[429] Moore EM, Wagner C, Komarnytsky S. The enigma of bioactivity and toxicity of botanical oils for skin care. Front Pharmacol 2020;11.
[430] Danby SG, AlEnezi T, Sultan A, Lavender T, Chittock J, Brown K, et al. Effect of olive and sunflower seed oil on the adult skin barrier: implications for neonatal skin care. Pediatr Dermatol 2013;30:42–50. https://doi.org/10.1111/j.1525-1470.2012.01865.x.
[431] Oláh A, Tóth BI, Borbíró I, Sugawara K, Szöllősi AG, Czifra G, et al. Cannabidiol exerts sebostatic and antiinflammatory effects on human sebocytes. J Clin Invest 2014;124:3713–24. https://doi.org/10.1172/JCI64628.
[432] Strunk T, Pupala S, Hibbert J, Doherty D, Patole S. Topical coconut oil in very preterm infants: an open-label randomised controlled trial. Neonatology 2017;113:146–51. https://doi.org/10.1159/000480538.
[433] Tietel Z, Melamed S, Eretz-Kdosha N, Guetta A, Gvirtz R, Ogen-Shtern N, et al. Anti-herpes simplex 1 activity of *Simmondsia chinensis* (jojoba) wax. Molecules 2021;26:6059. https://doi.org/10.3390/molecules26196059.
[434] Tang DYY, Yew GY, Koyande AK, Chew KW, Vo D-VN, Show PL. Green technology for the industrial production of biofuels and bioproducts from microalgae: a review. Environ Chem Lett 2020;18:1967–85. https://doi.org/10.1007/s10311-020-01052-3.
[435] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001.
[436] Marechal E. Biofuels from oleaginous algae principles, industry sectors, challenges. 2021.
[437] Durrett TP, Benning C, Ohlrogge J. Plant triacylglycerols as feedstocks for the production of biofuels. Plant J 2008;54:593–607. https://doi.org/10.1111/j.1365-313X.2008.03442.x.
[438] Radakovits R, Eduafo PM, Posewitz MC. Genetic engineering of fatty acid chain length in *Phaeodactylum tricornutum*. Metab Eng 2011;13:89–95. https://doi.org/10.1016/j.ymben.2010.10.003.
[439] Hao X, Chen W, Amato A, Jouhet J, Maréchal E, Moog D, et al. Multiplexed CRISPR/Cas9 editing of the long-chain acyl-CoA synthetase family in the diatom Phaeodactylum tricornutum reveals that mitochondrial ptACSL3 is involved in the synthesis of storage lipids. New Phytol 2022;233:1797–812. https://doi.org/10.1111/nph.17911.
[440] Blatti JL, Beld J, Behnke CA, Mendez M, Mayfield SP, Burkart MD. Manipulating fatty acid biosynthesis in microalgae for biofuel through protein-protein interactions. PLoS One 2012;7:e42949. https://doi.org/10.1371/journal.pone.0042949.
[441] Inaba Y, Nakahigashi K, Ito T, Tomita M. Alteration of fatty acid chain length of *Chlamydomonas reinhardtii* by simultaneous expression of medium-chain-specific thioesterase and acyl carrier protein. Phycol Res 2017;65:94–9. https://doi.org/10.1111/pre.12161.
[442] Sorigué D, Légeret B, Cuiné S, Blangy S, Moulin S, Billon E, et al. An algal photoenzyme converts fatty acids to hydrocarbons. Science 2017;357:903–7. https://doi.org/10.1126/science.aan6349.
[443] Moulin SLY, Beyly-Adriano A, Cuiné S, Blangy S, Légeret B, Floriani M, et al. Fatty acid photodecarboxylase is an ancient photoenzyme that forms hydrocarbons in the thylakoids of algae. Plant Physiol 2021;186:1455–72. https://doi.org/10.1093/plphys/kiab168.
[444] Imamura S, Kawase Y, Kobayashi I, Shimojima M, Ohta H, Tanaka K. TOR (target of rapamycin) is a key regulator of triacylglycerol accumulation in microalgae. Plant Signal Behav 2016;11:e1149285. https://doi.org/10.1080/15592324.2016.1149285.
[445] Sayer J, Sunderland T, Ghazoul J, Pfund J-L, Sheil D, Meijaard E, et al. Ten principles for a landscape approach to reconciling agriculture, conservation, and other competing land uses. Proc Natl Acad Sci USA 2013;110:8349–56. https://doi.org/10.1073/pnas.1210595110.
[446] Tilman D, Balzer C, Hill J, Befort BL. Global food demand and the sustainable intensification of agriculture. Proc Natl Acad Sci USA 2011;108:20260–4. https://doi.org/10.1073/pnas.1116437108.
[447] Spicher L, Glauser G, Kessler F. Lipid antioxidant and Galactolipid remodeling under temperature stress in tomato plants. Front Plant Sci 2016:7.
[448] Mühlroth A, Winge P, El Assimi A, Jouhet J, Maréchal E, Hohmann-Marriott MF, et al. Mechanisms of phosphorus acquisition and lipid class remodeling under P limitation in a marine microalga. Plant Physiol 2017;175:1543–59. https://doi.org/10.1104/pp.17.00621.
[449] Schwarz P, Herrfurth C, Steinem C, Feussner I. Lipidomics of *Thalassiosira pseudonana* as a function of valve SDV synthesis. J Appl Phycol 2022;34:1471–81. https://doi.org/10.1007/s10811-022-02715-0.
[450] Légeret B, Schulz-Raffelt M, Nguyen HM, Auroy P, Beisson F, Peltier G, et al. Lipidomic and transcriptomic analyses of *Chlamydomonas reinhardtii* under heat stress unveil a direct route for the conversion of membrane lipids into storage lipids. Plant Cell Environ 2016;39:834–47. https://doi.org/10.1111/pce.12656.
[451] Iwai M, Yamada-Oshima Y, Asami K, Kanamori T, Yuasa H, Shimojima M, et al. Recycling of the major thylakoid lipid MGDG and its role in lipid homeostasis in Chlamydomonas reinhardtii. Plant Physiol 2021;187:1341–56. https://doi.org/10.1093/plphys/kiab340.
[452] Zhang S, Zhang L, Xu G, Li F, Li X. A review on biodiesel production from microalgae: Influencing parameters and recent advanced technologies. Front Microbiol 2022:13.
[453] Altimari P, Di Caprio F, Brasiello A, Pagnanelli F. Production of microalgae biomass in a two-stage continuous bioreactor: control of microalgae-bacteria competition by spatial uncoupling of nitrogen and organic carbon feeding. Chem Eng Sci 2023;272:118604. https://doi.org/10.1016/j.ces.2023.118604.
[454] Wang T, Tian X, Liu T, Wang Z, Guan W, Guo M, et al. Enhancement of lipid productivity with a novel two-stage heterotrophic fed-batch culture of *Chlorella protothecoides* and a trial of CO2 recycling by coupling with autotrophic process. Biomass Bioenergy 2016;95:235–43. https://doi.org/10.1016/j.biombioe.2016.10.010.
[455] Gu C, Kim GB, Kim WJ, Kim HU, Lee SY. Current status and applications of genome-scale metabolic models. Genome Biol 2019;20:121. https://doi.org/10.1186/s13059-019-1730-3.
[456] Seaver SMD, Lerma-Ortiz C, Conrad N, Mikaili A, Sreedasyam A, Hanson AD, et al. PlantSEED enables automated annotation and reconstruction of plant primary metabolism with improved compartmentalization and comparative consistency. Plant J 2018;95:1102–13. https://doi.org/10.1111/tpj.14003.
[457] Belcour A, Got J, Aite M, Delage L, Collén J, Frioux C, et al. Inferring and comparing metabolism across heterogeneous sets of annotated genomes using AuCoMe. Genome Res 2023;33:972–87. https://doi.org/10.1101/gr.277056.122.
[458] Salek RM, Conesa P, Cochrane K, Haug K, Williams M, Kale N, et al. Automated assembly of species metabolomes through data submission into a public repository. GigaScience 2017;6:gix062. https://doi.org/10.1093/gigascience/gix062.
[459] Frainay C, Schymanski EL, Neumann S, Merlet B, Salek RM, Jourdan F, et al. Mind the gap: mapping mass spectral databases in genome-scale metabolic networks reveals poorly covered areas. Metabolites 2018;8:E51. https://doi.org/10.3390/metabo8030051.
[460] Bernstein DB, Sulheim S, Almaas E, Segrè D. Addressing uncertainty in genome-scale metabolic model reconstruction and analysis. Genome Biol 2021;22:64. https://doi.org/10.1186/s13059-021-02289-z.
[461] Huckauf J, Nomura C, Forchhammer K, Hagemann M. Stress responses of Synechocystis sp. strain PCC 6803 mutants impaired in genes encoding putative alternative sigma factors. Microbiology 2000;146:2877–89. https://doi.org/10.1099/00221287-146-11-2877.
[462] Core Writing Team. In: Pachauri RK, Meyer LA, editors. IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland: IPCC; 2014.
[463] Yang LH, Rudolf VHW. Phenology, ontogeny and the effects of climate change on the timing of species interactions. Ecol Lett 2010;13:1–10. https://doi.org/10.1111/j.1461-0248.2009.01402.x.
[464] González-Fernández C, Le Grand F, Bideau A, Huvet A, Paul-Pont I, Soudant P. Nanoplastics exposure modulate lipid and pigment compositions in diatoms. Environ Pollut 2020;262:114274. https://doi.org/10.1016/j.envpol.2020.114274.