Supporting information
Integrated extraction and catalytic upgrading of microalgae lipids in supercritical carbon dioxide

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1 General considerations

Methanol was distilled over Mg under a nitrogen atmosphere and stored over molecular sieves. All other solvents were standard analytical grade and used as received. CO (N3.7), CO_2 (N4.5) (used with a dip-tube tank for the Supercritical fluid extractor SFX-110W) and CO_2 (N3.5) (used for the high-pressure reactor) were purchased from Lindegas and used as received. Ethylene (N4.5) was supplied by Air Liquide. Methyl oleate (Dakolub MB9001), was kindly donated by DAKO AG. It was distilled before use and stored under an inert atmosphere. The methyl oleate (92.5%) used contains small amounts of methyl palmitate (2.5%), methyl stearate (1.5%) and methyl linoleate (2.5%). Eicosapentaenoic acid (EPA) and Hoveyda-Grubbs 2nd generation catalyst were purchased from Carbosynth. EPA was distilled in vacuum prior to use. Methyl palmitate was purchased from Nu-Check Prep, Inc. Genuine standards of pigments were bought by DHI. *Cis/trans*-2-butene (99%) and α,α'-bis(di-*tert*-butylphospino)xylene (dtpbx) originates from ABCR GmbH. 1,4-Cyclohexadiene was purchased from Acros Organics. Hoveyda-Grubbs 1st generation catalyst, sulfuric acid, ethyl vinyl ether, 1-decene, 1-octene, 5-hexenoic acid and 9-decenoic acid were purchased from Sigma Aldrich. Diacylglyceride standards (99%) were bought from Avanti Lipids. 1,17-dimethyl heptadecanedioate and 1,19-dimethyl nonadecanedioate were prepared by carbonylation of 16:1 and 18:1. 1,21-dimethyl heneicosanedioate[1] and the Pd-complex [Pd(dtbpx)$(OTf)_2$][2] were prepared following reported synthetic procedures.

After harvest, wet and dried microalgal cells and all extracts were protected from light to prevent oxidation.

2 High-pressure instrumentation

The high-pressure reactor (Figure S1) is a variable volume view cell, built and designed by New Ways of Analytics (NWA GmbH) in Lörrach and equipped with a pneumatic compressor (PM-101), overhead stirrer, pressure gauge (0-1000 bar), pneumatically operated relieve valve at the bottom of the reactor that connects to a steal depressurization cylinder, and an internal thermocouple that controls electric heating cartridges in the reactor wall. The volume of the reactor is 60 mL, which can be varied down to 30 mL pneumatically. CO_2 was supplied to the reactor via cryogenic SCF pumps (NWA GmbH).

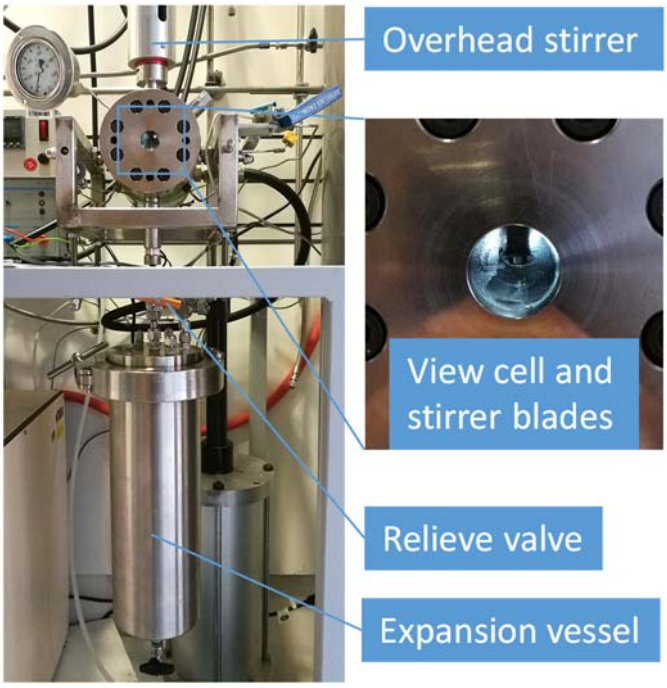


Figure S1 High-pressure reactor employed**.**

A supercritical fluid extractor SFX-110W (Figure S2) from Supercritical Fluid Technologies was utilized. This reactor is supplied with a $scCO_2$ stream by a piston pump, which is fed with liquid CO_2 from a dip-tube tank. A 10 mL and 100 mL vessel, respectively, were used as extraction compartments. Their volume could be reduced and adjusted by addition of glass beads.

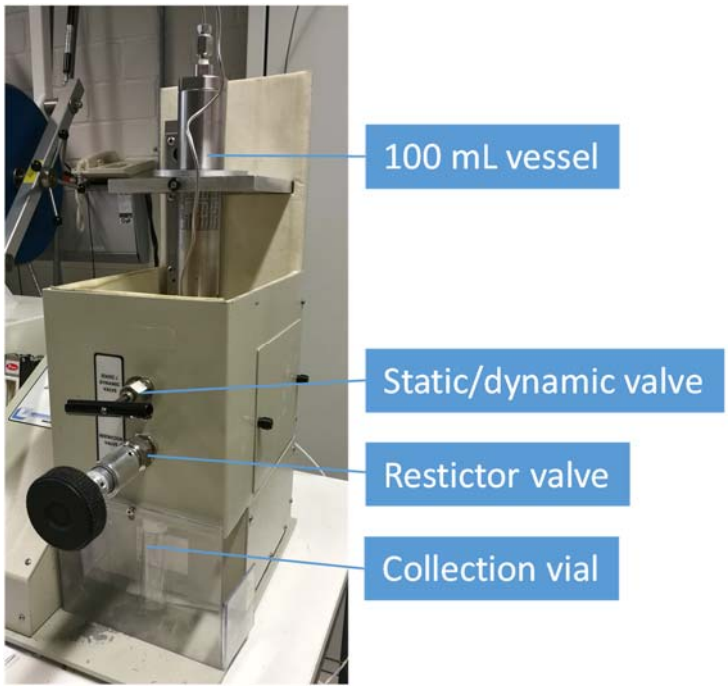


Figure S2 Supercritical fluid extractor SFX-110W.

3 Algae cultivation and harvest

3.1 Algae cultivation

The examined strain is a single clone colony of *Phaeodactylum tricornutum* (*P. tricornutum*) wildtype UTEX 646 (WT4). WT4 was stored on agarose plates at 20 °C in a room with day-night rhythm (16/8) at light intensities of around 20 µmol/m^2/s. 35 mL of pre-precultures were inoculated in triplicates in f2-medium[3] and grown for 14 days at 18 °C with permanent light and a light intensity of 55-65 µmol/m^2/s while shaking with 850 rpm in order to let the cells adapt to the new growth conditions.

In the next step, the pre-precultures were used to inoculate 400 mL of the precultures (triplicates). These precultures were also cultivated at 18 °C in permanent light of 55-65 µmol/m^2/s shaking with 850 rpm. After 5-6 days, when the cells were in the exponential growth phase (measured cell count: 1.6-2.6 x 10^6 cells/mL), 10 L round bottom flasks were inoculated with a cell count of 40 000 cells/mL. The cultures were cultivated for 5-6 weeks (late stationary growth phase) at 18 °C in permanent light of 55-65 µmol/m^2/s and aerated with ambient air through a sterile filter. Cells were counted with a Multisizer 3 coulter counter from Beckman Coulter, Pasadena, California, USA.

3.2 Harvest

Stationary cultures were harvested with cell counts between 6.2-14.4 x 10^6 cells/mL (5-6 weeks), as determined with the Multisizer 3 coulter counter. In order to ensure comparability between the different extraction methods, every 10 L-culture was separated into three aliquots (Figure S3). One aliquot was extracted via the method established by Folch *et al.*[4] (Folch extraction), one was extracted with supercritical CO_2 ($scCO_2$ extraction) and the last one was extracted via soxhlet extraction in hexane (Hexane Extraction), which is not part of this contribution.

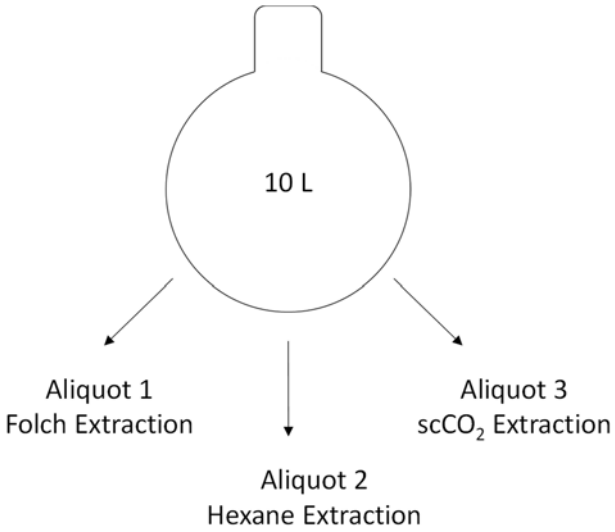


Figure S3 Aliquotation of 10 L round bottom flasks for microalgal oil analytics.

Cells were harvested in two centrifugation steps. The first pre-concentration step was performed either with a Sorvall RC 6 centrifuge with a Sorvall SLA 3000 rotor (5000 g, 10 min at 4 °C) from Thermo Fisher Scientific (Waltham, Massachusetts, USA) or a Contifuge® Stratos with a titanium rotor (5000 g, 4 °C) from Hereaus (Hanau, Germany). The second centrifugation step was conducted with an Allegra 25R centrifuge (5000 g, 10 min at 4 °C) from Beckman coulter. The residual water was discarded and the wet weight was determined gravimetrically (ME36 S microscale from Sartorius, Göttingen, Germany). After freeze-drying (ALPHA 2-4 LD plus from Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) the dry weight was determined gravimetrically (ME36S microscale from Sartorius) and the cells were stored in the dark at -20 °C.

Optionally, the wet algae were pre-treated by ultrasonication (see Figure S4) for 10 min with an on/off pulse of 10 s and amplitude of 60%, using an ultrasound homogenizer HD3200 from BANDELIN with a KE76 sonotrode, prior to freeze-drying. After freeze-drying they were crushed mechanically with a pestle and mortar.

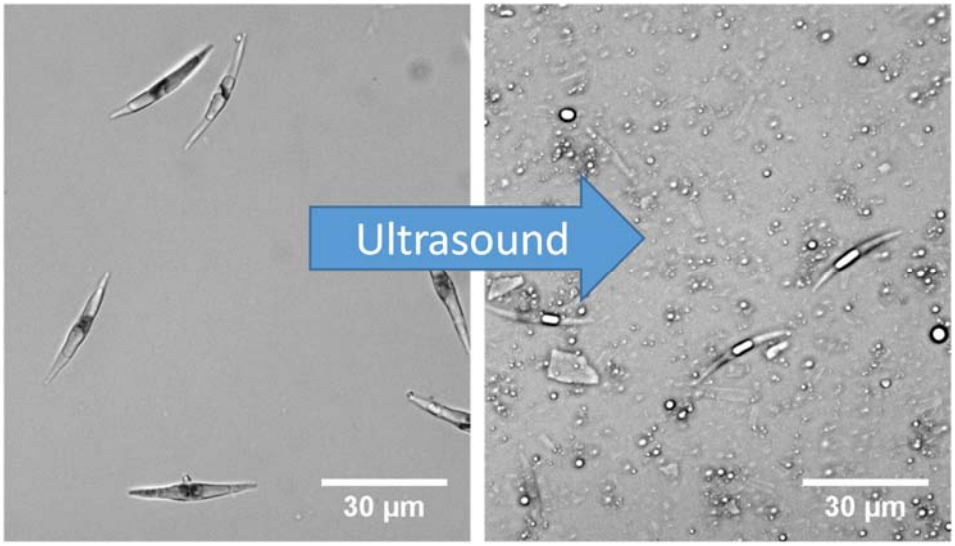


Figure S4 Light microscopy images of *P. tricornutum* before (left) and after cell disruption by ultrasound (right). The images were recorded on an Olympus BX 51 equipped with a Zeiss AxioCam MRm. Visible droplets can be attributed to intracellular and extracellular lipid droplets.

3.3 Analysis of dried algae

The residual water content, the amount of inorganic and organic matter of the freeze-dried microalgae was determined via thermogravimetric analysis (TGA) in triplicates, on a Jupiter STA449F3 instrument from Netzsch, Selb, Germany. The sample was heated up slowly to 100 °C with 2 K/min, and more rapidly to 1000 °C with 30 K/min in a gas mixture of 85 % O_2 and 15 % N_2. The data was analyzed by Netzsch Proteus Thermal Analysis software.

For selected samples also the relative composition of lipids to carbohydrates to proteins was determined via Fourier-transform infrared spectroscopy (FT-IR). The freeze-dried cells were dissolved in water with a concentration of 15 µg/µL and 2 µL were applied on a 384 well silica plate and dried at 38 °C for 30 min. IR spectra were recorded with a Bruker Tensor II with a HTS-XT microplate reader module for high-throughput screening with a 384 well silica plate (see chapter 5.4). The acquired IR spectra were analyzed with Origin 2015G of Origin Lab Cooperations. The height of the signals at 1740 cm^{-1}, 1545 cm^{-1} and 1150 cm^{-1} were determined and the content of biomolecules (lipid, protein, glyceride – carbohydrates and glycerol) was calculated with the respective calibration curves (Table S6) and normalized to 100 %.

Table S1 Averaged composition of dried biomass (% of dry weight) before extraction measured via TGA and depicted in the first three rows. The mean ± standard deviation from 3 independent biological replicates is given. The composition of the organic matter (glyceride, protein, lipid) is determined via FT-IR (error ~2%) and normalized to 100 %.

TGA			FT-IR		
Water	Inorganic matter	Organic matter	Glyceride[a]	Protein[a]	**Lipid[a]**
4.1 ± 1.2 %	7.4 ± 2.3 %	88.5 ± 2.2 %	13.3 %	60.7 %	**26.1 %**

[a] Relative composition normalized to 100 %.

The FT-IR measurement of dried algae cells (Table S1) allows us to determine the efficiency of the extractions. From extraction according to Folch[4] 28% of dry weight were extracted, while $scCO_2$ at optimized conditions yielded 25% of dry weight. This corresponds to almost quantitative extraction, as a lipid content in the dried algae cells of 26.1 wt% was determined from FT-IR measurements.

4 Extraction methods

4.1 Organic solvent extraction

Organic solvent extraction was performed according to Folch *et al.*[4] The dried cells were suspended in a mixture of $CHCl_3$/MeOH (2:1) in a 100 mL round-bottom flask, installed in an ice bath, and ultrasonicated with a HD3200 ultrasonicator (BANDELIN electronic GmbH & Co. KG) with a KE76 sonotrode for 10 min with an on/off pulse of 10 s and an amplitude of 60 %. After addition of MilliQ ($CHCl_3$/MeOH/MilliQ 8:4:3), phases were separated with a separation funnel. The upper water-MeOH phase containing free carbohydrates, proteins and other hydrophilic compounds, was extracted three times with $CHCl_3$. The combined $CHCl_3$ phases were dried over $MgSO_4$. The solvent was evaporated and the oil dried in vacuum. The obtained algae oil was stored at -20 °C.

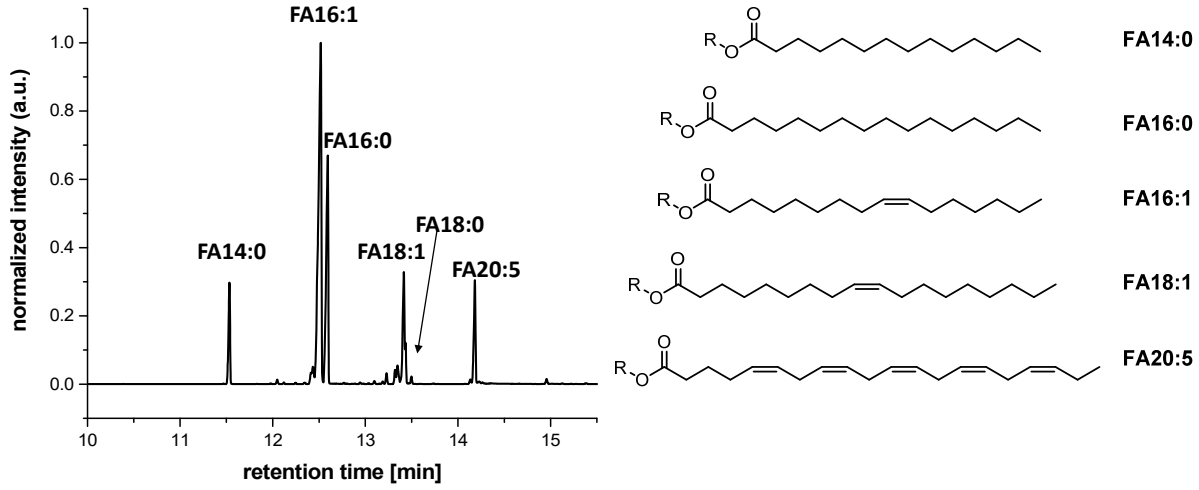


Figure S5 Gas chromatogram of algae oil from *P. tricornutum* extracted via the method of Folch.[4] The sample was transesterified with methanol for gas chromatography.

4.2 Supercritical CO_2 extraction

4.2.1 General procedure for $scCO_2$ extraction

In a 5-micron mesh nylon sample bag, 1 g freeze dried algae or ultrasound pre-treated freeze-dried algae, respectively, was placed. The bag was closed and positioned in a 10 mL stainless steel extraction vessel with filter disks at both openings. The empty volume was filled up with glass beads. The vessel was placed in the extractor (Figure S2), pressurized and heated. For optimization of $scCO_2$ extraction different pressures were applied (see Table S2). For characterization, the algae oil was extracted at 413.7 bar (6000 psi). The algae were allowed to soak for 20 minutes. Subsequently, the algae oil was extracted employing a 10 mL/min flow of liquid CO_2 (dual syringe pump read-out, model SFT10 from Supercritical Fluid Technologies) for 20 minutes. The soaking and flow extraction were each repeated four times. The extract was collected behind the backpressure valve, the CO_2 being evaporated upon expansion. The extracted microalgae oil was stored at -20 °C.

4.2.2 Optimization of $scCO_2$ extraction

Table S2 Conditions and yields of $scCO_2$ extracted algae oil from freeze-dried algae of the strain *P. tricornutum*.

Entry	Pressure [bar]	Temperature [°C]	Density [g/mL][a]	Yield [wt%][b]
1	310	40	0.915	15
2	310	45	0.896	15
3	310	60	0.837	16
4	414	45	0.945	18
5	414	60	0.897	17
6	517	45	0.982	13
7	517	60	0.939	19
8	517	75	0.897	20
9	621	90	0.898	19
10	310	45	0.896	20
11	414	60	0.897	21
12	517	75	0.897	21
13	621	90	0.898	25

10 mL vessel, 1 g freeze-dried (entry 1-9) or ultrasound pre-treated freeze-dried algae (entry 10-13), temperature of restrictor block 60 °C, four times 20 min soak and subsequent 20 min extraction with 10 mL/min CO_2.[a]Density of neat carbon dioxide at the given temperatures and pressures were determined according to Ref 5. [b] Determined by GC with dodecane as an internal standard after transesterification with methanol.

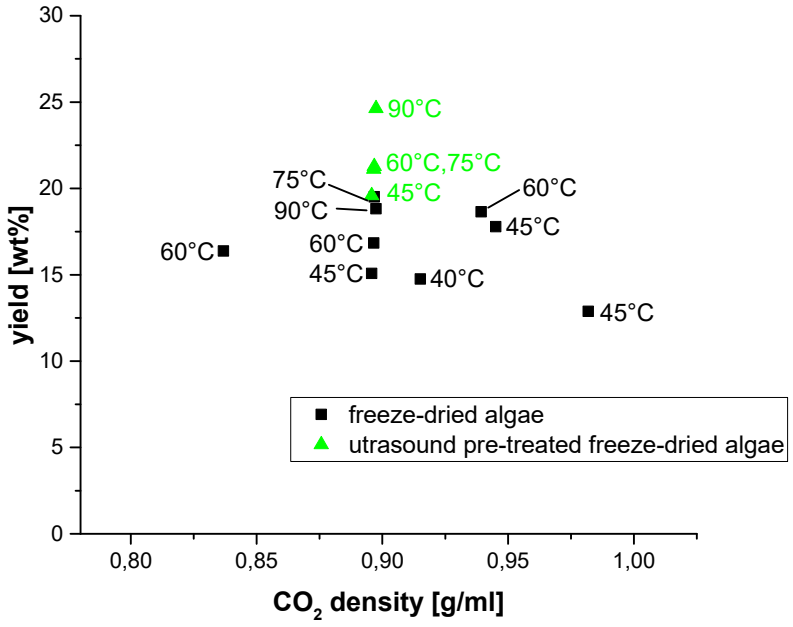


Figure S6 Yields of extraction of algae oil from *P. tricornutum* with $scCO_2$ at different densities. Corresponding pressures are given in Table S2.

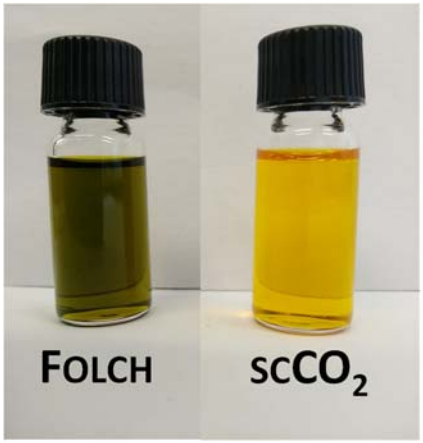


Figure S7 Appearance of algae oils extracted according to Folch and with $scCO_2$, respectively, diluted in CH_2Cl_2, showing the differences in pigment extraction. The Folch extraction also extracts chlorophylls, while $scCO_2$ extracts carotenoids selectively (cf. section 5.5).

5 Analysis of extracted algae oils

5.1 Overview of components in microalgae oil

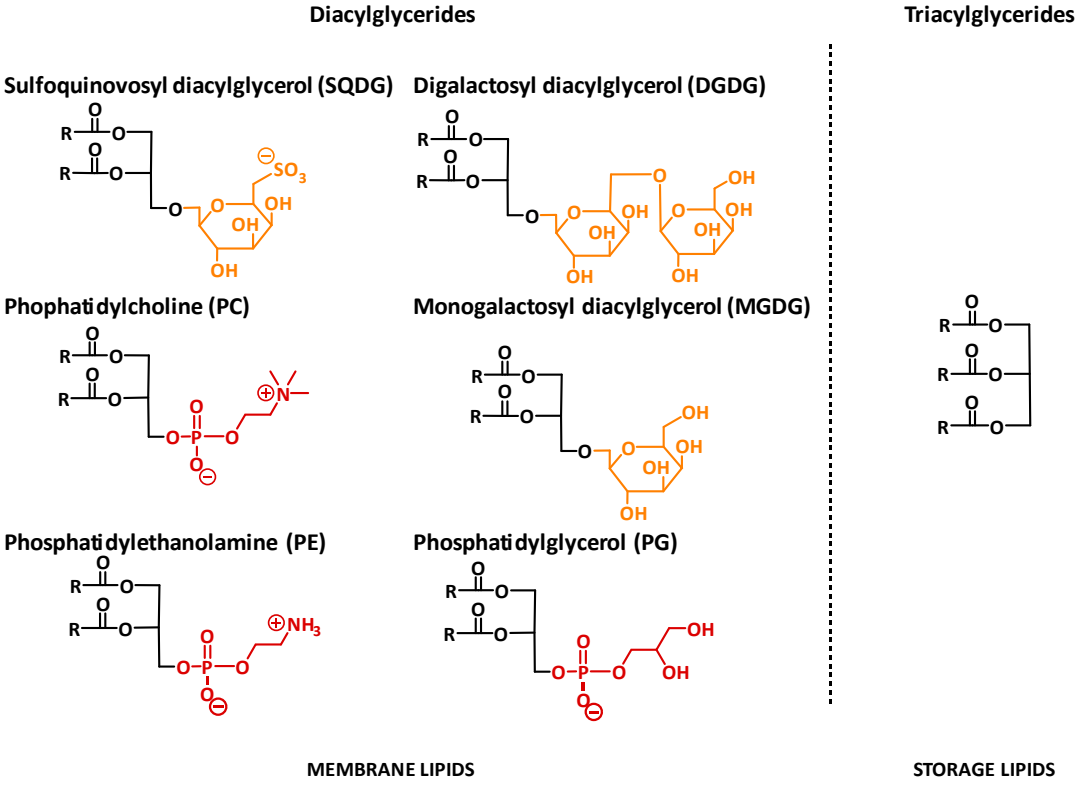


Figure S8 Lipid classes found in *P. tricornutum*. Carbohydrate moieties present in glycolipids are highlighted in orange. Phosphorous/nitrogen containing moieties present in phospholipids are highlighted in red. R – fatty acid residue, depicted in Figure S9.

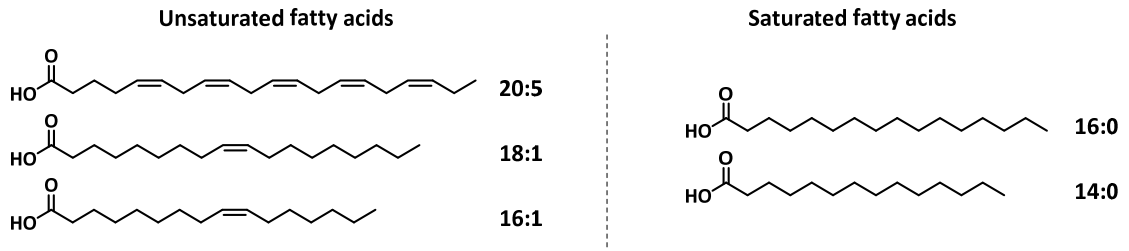


Figure S9 Fatty acids in *P. tricornutum*.

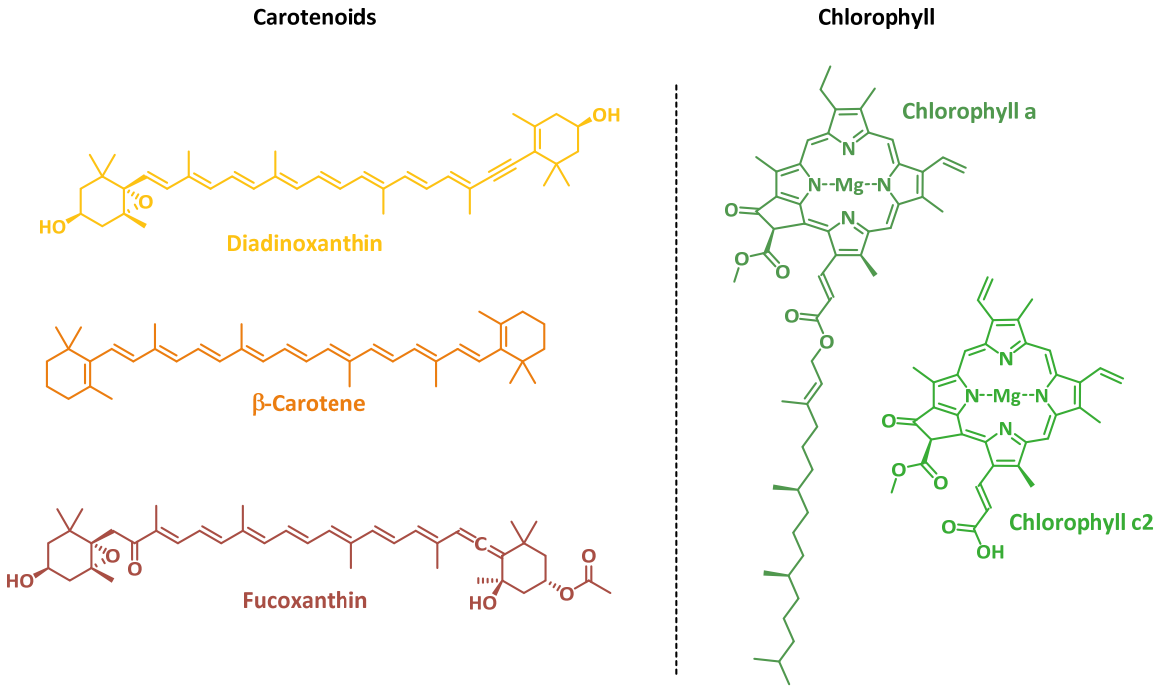


Figure S10 Pigments in *P. tricornutum* divided between carotenoids and chlorophylls. The colors represent the color of the respective pigment.

5.2 Gas chromatography (GC)

Gas chromatography was carried out on a PerkinElmer Clarus 500 instrument with an autosampler and FID detection on a PerkinElmer Elite-5 (5% Diphenyl- 95% Dimethylpolysiloxane) Series Capillary Column (Length: 30 m, Inner Diameter: 0.25 mm, Film Thickness: 0.25 mm), using helium as the carrier gas at a flow rate of 1.5 mL min^{-1}. The injector temperature was 300 °C and the detector temperature 280 °C. For the analysis of the fatty acid composition in algae oil or the product analysis after metathesis or alkoxycarbonylation two different programs were used. 1. Fatty acids in algae oil and for carbonylation product analysis: the oven was kept isothermal at 90 °C for 1 min after injection, then heated with 30 °C/min to 280 °C, and kept isothermal at 280 °C for 8 min. 2. For metathesis product analysis: the oven was kept at 50 °C for 3 min, then heated with 20 °C/min to 280 °C, and kept

isothermal at 280 °C for 5 min. For both methods the split was defined as a 20 ml min^{-1} inlet flow and 1.5 ml min^{-1} column flow.

All algae oil samples were weighed in on a ME 36S microscale and esterified before GC analysis for 4 days at 50 °C with 1 mL of a 1 % methanolic H_2SO_4 solution, also containing an internal standard (0.815 mg/mL) for quantification.

The fatty acid esters and metathesis or alkoxycarbonylation products were identified via comparison of retention times and enrichment experiments with commercially available genuine standards.

5.3 Thin-layer chromatography (TLC)

The separation of polar diacylglycerides (DAG), their identification and quantification were performed using thin-layer chromatography (TLC) on high-performance silica gel plates (HPTLC, Merck, Darmstadt, Germany). Each sample was separated with two different eluent systems to enable quantification of all polar diacylglycerides (Ventrella *et al.*[6] and Mock and Kroon[7] system, Table S3).

Table S3 Eluent systems for lipid determination and quantification.

Mock and Kroon	
Solvent	**Volume [mL]**
methyl acetate	25
isopropanol	25
chloroform	25
methanol	10
0.25 % KCl (w/v)	4
Ventrella *et al.*	
chloroform	75
methanol	13
acetic acid glacial	9
ddH_2O	3

Two solvent systems were necessary, because of the chlorophyll pigments, which show the same retention characteristic as monogalactosyl diacylglycerol (MGDG) in the Mock and Kroon eluent (Table S12, black spots at running front). In the Ventrella system phosphatidylcholine (PC) and digalactosyl diacylglycerol (DGDG) are not separable. Every run was carried out with a calibration standard mixture (in the following referred as MIX) consisting of a mixture of neat samples of all DAG (Table S4), which was applied in triplicates to enhance reliability (Figure S12).

Prior to a run, every plate was dried for 60 min at 120 °C. Meanwhile, the chambers were equilibrated with the solvent mixtures. Samples and standard triplicates were applied with a Hamilton-syringe. Each sample was dissolved in EtOH to a concentration of around 1 mg/mL. A blow dryer was installed above the plate to accelerate evaporation of the solvent. The R_f-values and detection limit of the respective DAG were determined by concentration dependent runs of neat samples in both solvent systems. To achieve optimal separation, the running length for the Mock and Kroon system was 8 cm, and 7 cm for the Ventrella system.

After the run, the plates were dried for 5 min at 120 °C. The plates were stained with 0.05 % primuline (Sigma Aldrich) in 80 % acetone/H_2O applied with a TLC sprayer and dried at room temperature according to White *et al*.[8] Documentation was performed via the fluorescence of the primuline, detected by a Luminescent Image Analyzer Amersham Imager 600 from GE Healthcare Biosciences-AB, Chicago, Illinois, USA. The plates were semi-quantitatively evaluated by Image Studio Lite 5.2.5 of LI-COR Biosciences, Lincoln, Nebraska, USA. The pixel intensity linearly correlates to the lipid content and is normalized to the amount of the respective component in the MIX.

Table S4 R_f-values in the different eluent systems and concentration of the respective components in the standard mixture (MIX). DGDG – digalactosyl diacylglyceride, MGDG – monogalactosyl diacylglyceride, PC – phosphatidylcholine, PE – phosphatidylethanolamine, PG – phosphatidylglycerol, SQDG – sulfoquinovosyl diacylglyceride, TAG – triacylglyceride.

Eluent system	Lipid	R_f-value	Concentration of MIX components for calibration [mg/mL]
Ventrella *et al.*	MGDG	0.8	171.0
	PE	0.4	174.2
	PG	0.2	178.0
Mock and Kroon	DGDG	0.4	169.4
	SQDG	0.3	168.0
	PC	0.2	166.8
	TAG	1	170.4

TLC substantiates that $scCO_2$ is not able to extract polar diacylglycerides. No signals can be detected (Figure S12). The quantitative evaluation of four independent TLC measurements show that algae oil extracted according to Folch contains 11% of polar diacylglycerides. The composition of these polar diacylglycerides is depicted in Figure S11 and well comparable to reported data from Yang *et al*.[9]

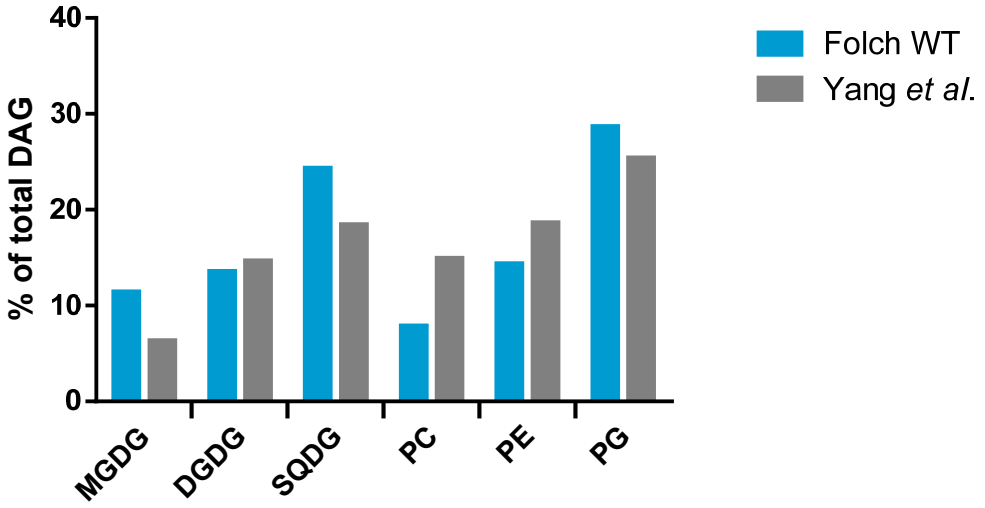


Figure S11 Polar diacylglyceride (DAG) composition *P. tricornutum* extracted according to Folch (blue columns) and compared to reported data for comparable oils from Yang *et al*. (dark grey columns).[9] In $scCO_2$ extraction no DAG are found.

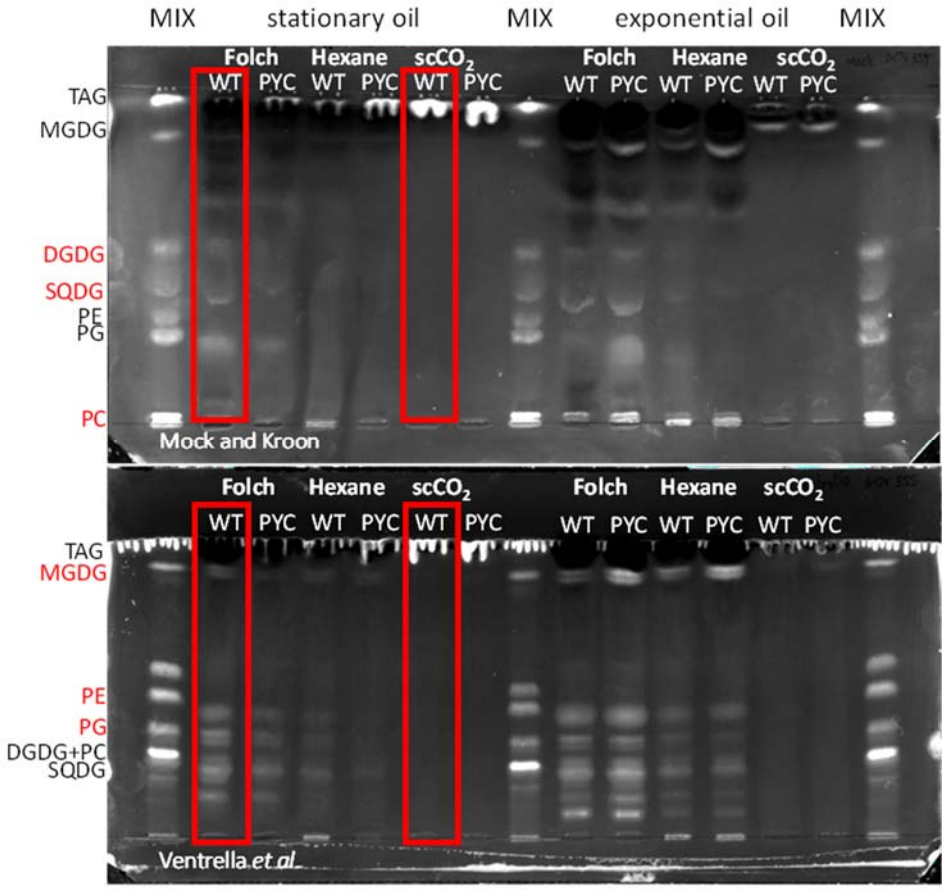


Figure S12 Exemplary TLC plates with all samples, separated in the two solvent systems (Mock and Kroon – top, Ventrella – bottom). The polar diacylglycerides, which can be detected with the respective eluent system are marked in red. Important for this study are only the lines marked in red. DGDG – digalactosyl diacylglyceride, MGDG – monogalactosyl diacylglyceride, PC – phosphtidylcholine, PE – phosphatidylethanolamine, PG – phosphatidylglycerol, SQDG – sulfoquinovosyl diacylglyceride, TAG – triacylglycerol, MIX – mixture of MGDG, DGDG, SQDG, PC, PE, PG and TAG with the concentration of ~170 mg mL^{-1}.

5.4 Fourier-transform infrared spectroscopy (FT-IR)

FT-IR was measured with a Bruker Tensor II with a HTS-XT microplate reader module for high throughput screening with a 384 well silica plate. Infrared spectra were recorded in transmission mode with 32 scans co-added to enhance the signal-to-noise ratio, in the range 4000–700 cm^{-1} (Software: OpusLab 5.0 software; Bruker Optics, Ettlingen, Germany) and at a spectral resolution of 4 cm^{-1}. For detection a deuterated triglycine sulphate (DTGS) detector was used. A Blackman–Harris 3-term apodization function was applied to minimize artificial side maxima due to Fourier transformation, with a zero-filling factor of 2. The pinhole aperture was set to 3 mm. The measurements were conducted in the laboratories and according to the reported procedure[10] of the group of Prof. Christian Wilhelm at the University of Leipzig. Calibration was kindly provided by Dr. Heiko Wagner from the Wilhelm group with glycerol tripalmitate as lipid standard, BSA as protein standard and glucose as carbohydrate standard. The calibration is depicted in Table S6.

Measurement of neat algae oil resulted in total absorption, therefore dilution with $CHCl_3$ to a concentration of 13.3 µg/µL was conducted. Each sample (0.5 µL) was measured in five to eleven technical replicates. Figure S13 and Table S5 list the assignment of the samples. Please note that the right (A to P) and bottom (1 to 24) labeling of the plate is used here. We see some coffee stain effects, which can impede repeatablity. To increase the data quality, additional technical replicates (>5) were conducted.

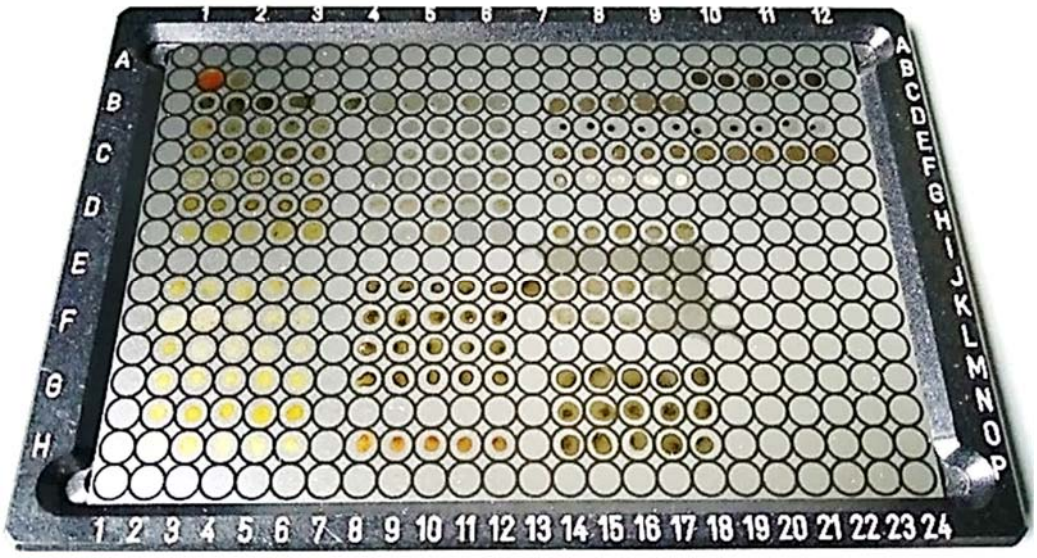


Figure S13 Silica plate with algae oil samples, always applied in >5 technical replicates. The right (A to P) and bottom (1 to 24) labeling applies here.

Table S5 Assignment of algae oils to samples on the silica plates, depicted in Figure S13. For the results discussed here only samples marked red are important.

silica plate	sample	silica plate	sample	silica plate	sample
C2-C5 + C7	Folch WT stat n1-n3	C8-C12	$scCO_2$ WT stat n1-n3	C14-C18	Hexane WT exp n2
D2-D6		D8-D12		D14-D18	Hexane exp n1-n2
E2-E6		E8-E12		E14-E18 E19-E23	
F2-F6		F8-F12		F14-F18 D19-D23	Hexane WT exp n3
G2-G6		G8-G12		G14-G18	-
H2-H6		H8-H12		H14-H18	$scCO_2$ WT exp n1-n2
I2-I5	-	I8-I12	-	I14-I18	
J2-J5	Hexane WT stat n1-n3	J8-J12 M14-M18	Folch WT exp n1-n2	J14-J18	$scCO_2$ exp n1-n2
K2-K6		K8-K12 N14-N18		K14-K18	
L2-L6		L8-L12 O14-O18	Folch exp n1-n2	L14-L18	-
M2-M6	Hexane stat n1-n3	M8-M12 B19-B23			
N2-N6		N8-N12	-		
O2-O6		O8-O12	Hexane WT exp n1		

The C-O stretch vibration at 1740 cm^{-1} correlates with the lipid concentration in the sample, C-O stretch vibration of the amide (amide II) at 1545 cm^{-1} correlates with the protein concentration and C-O-C vibration at 1150 cm^{-1} correlates with the glyceride (carbohydrate and glycerol) concentration.

The lipid concentration was calculated from the height of the lipid signal at 1740.8 cm^{-1} and the calibration curve (Table S6).

Table S6 Calibration curves of reference substances and coefficient of determination (R^2). y – signal height, x – concentration, a – slope.

Oil component	Wave number	Reference substance	a from y = ax	R2
Lipid	1740 cm^{-1}	Glycerol tripalmitate	0.0172	0.977
Protein	1545 cm^{-1}	BSA	0.083	0.994
Carbohydrate	1150 cm^{-1}	Glucose	0.00295	0.997

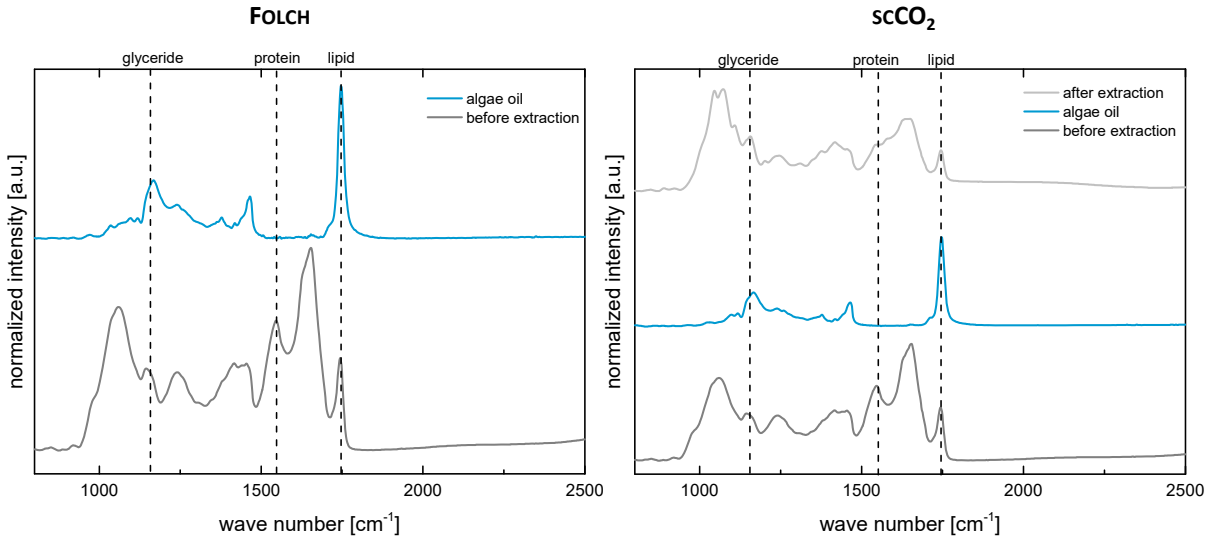


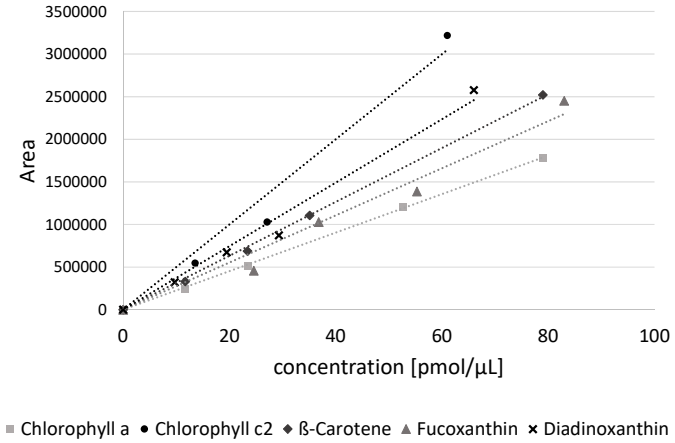
Figure S14 FT-IR spectra of algae oil extracted according to Folch (left) and with $scCO_2$ (right), respectively, and of *P. tricornutum* biomass prior to and after extraction.

FT-IR spectra were recorded from the dried biomass prior to extraction (Figure S14 bottom, dark grey), the algae oil (Figure S14 middle, blue) and in case of $scCO_2$ extraction also the biomass residue after extraction (Figure S14 top, light grey), showing us the efficiency of $scCO_2$ extraction. As can be seen there is some lipid signal left, most likely corresponding to polar diacylglycerides, which cannot be extracted with $scCO_2$. Polar diacylglycerides comprise in phospholipids and glycolipids (Figure S8), of which the latter account for the carbohydrate signals in algae oil together with glycerol esters. The lower intensity glyceride band for $scCO_2$ extracted oil qualitatively agrees with the observed absence of polar diacylglycerides found by TLC (cf. section 5.3). The C-O stretch vibration of the amide corresponding to the proteins is not found in both algae oils (Figure S14 middle, blue). $ScCO_2$ is very selective for triacylglyceride extraction.[11]

5.5 High-performance liquid chromatography (HPLC)

Analytical high-performance liquid chromatography (HPLC) was conducted on a ELITE LaChrom system (Elite LaChrom Organizer, pumps *L-2130*, auto sampler *L-2200*, column thermostat Jetstream 2 PLUS, diode array detector *L-2455* and software *EZChrom Elite* version 3.2.1 from Agilent, Santa Clara, California, USA) from VWR Hitachi now Avantor Performance Materials LLC (Darmstadt, Germany) using a EC 250/4 Nucleosil 300-5 C18 column (length: 250 mm, inner diameter: 4 mm) from Macherey-Nagel (Düren, Germany) in an oven kept at 30 °C. The HPLC was calibrated with genuine standards of chlorophyll a, chlorophyll c2,

diadinoxanthin, fucoxanthin, β-carotene (Figure S15). Concentrations between 0.25-2 pmol/µL were measured. A volume of 40 µL of sample was injected.



	a	R^2
■ Chlorophyll a (430 nm)	22614	0.999
● Chlorophyll c2 (440 nm)	49954	0.974
♦ ß-Carotene (480 nm)	31626	0.998
▲ Fucoxanthin (440 nm)	27665	0.973
x Diadinoxanthin (480 nm)	37270	0.984

Figure S15 Calibration curves of reference pigments and the standard coefficient of determination.

The separation was accomplished with three solvents and a flow rate of 0.8 mL/min. The gradient elution programming (Table S7 and Figure S16) was established in the group of Prof. Peter Kroth.[12]

Table S7 Gradient profile for pigment separation via HPLC. A – 85 % MeOH/15 % 0.5 M NH_4CH_3COO, B – 90 % acetonitril/10 % H_2O, C – 100 % ethylacetate.

Time	A	B	C
0	60	40	0
2	0	100	0
7	0	90	10
21	0	60	40
25	0	30	70
28.5	0	30	70
29.5	0	100	0
30.5	60	40	0

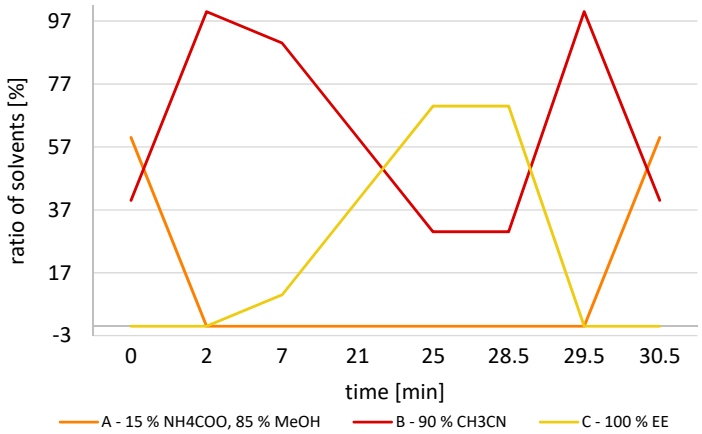


Figure S16 Gradient profile of the HPLC separation method. A – 85 % MeOH/15 % 0.5 M NH_4CH_3COO, B – 90 % acetonitrile/10 % H_2O, C – 100 % ethyl acetate.

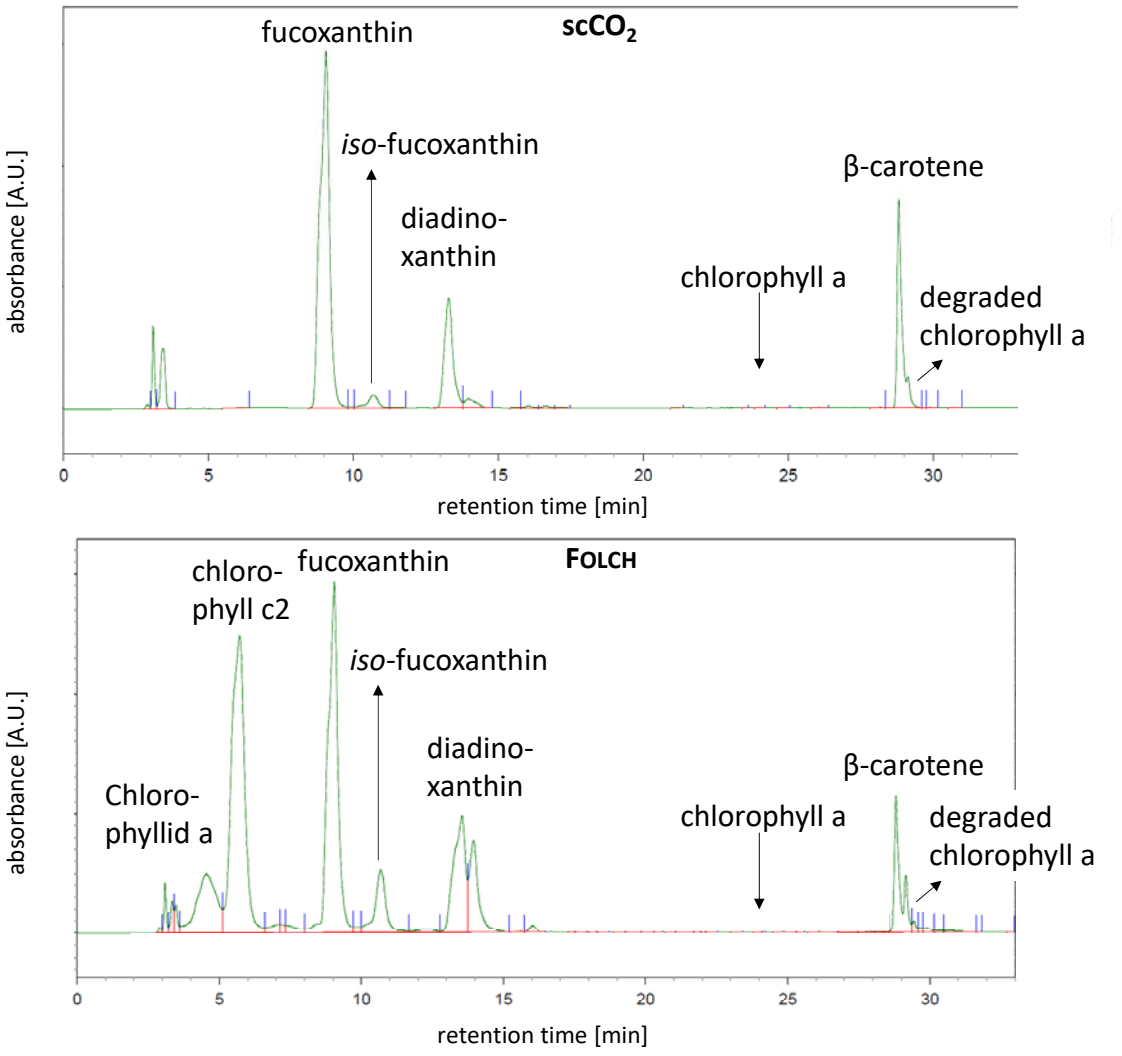


Figure S17 Typical chromatogram of algae oil extracted according to Folch (bottom) and with $scCO_2$ (top). Oil extracted by $scCO_2$ does not contain polar chlorophylls. Chlorophyll a is not found in both oils, as it is degraded by the highly active chlorophyllase a in *P. tricornutum*.[13,14]

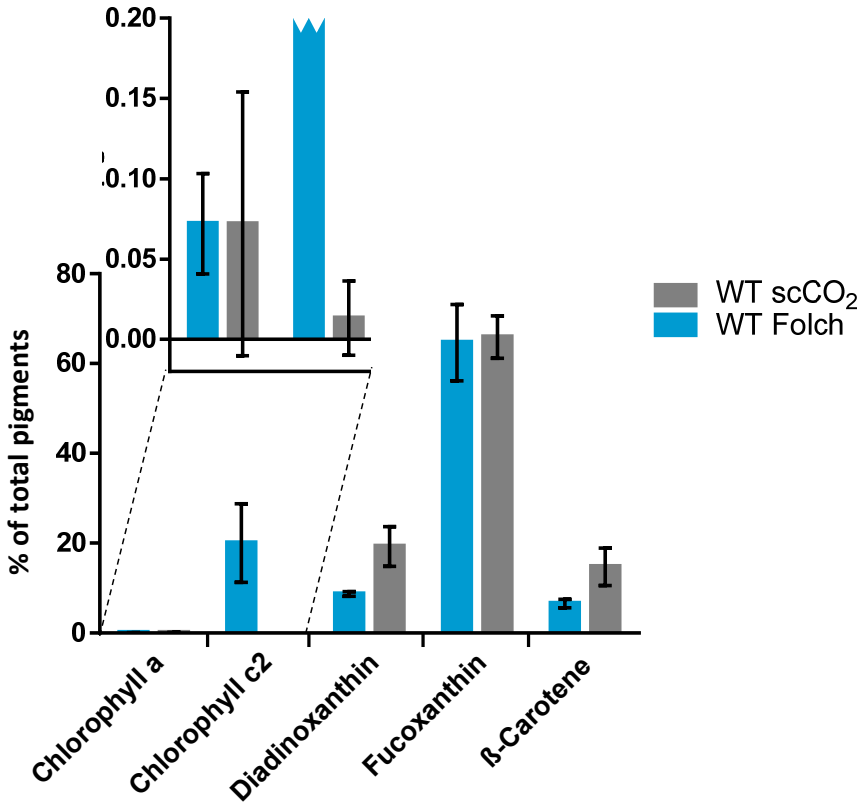


Figure S18 Composition of pigments in *P. tricornutum*. Pigments account for 2.4 wt% of algae oil extracted according to Folch, and 1.0 % of the oil extracted by $scCO_2$. Depicted are the means ± standard deviation. Inlets, show a magnification to the low chlorophyll content. Comparison was carried out using two-way ANOVA with Tukey´s multiple comparisons post-test.

5.6 Statistical analysis

Statistical analysis was carried out using Prism 6.07 from GraphPad Software, Inc. Results were always given as mean ± standard deviation with three biologically independent experiments. Multiple comparisons were performed using one-way ANOVA with a Tukey-Kramer or Dunnett´s post-test or two-way ANOVA with a Tukey or Sidak´s multiple comparisons post-test.

5.7 Summary of algae oil composition

Table S8 Overview of results from all analytical methods used. For TLC no standard deviation is given, as it is a semi-quantitative method.

Folch	Fatty Acids	Lipids (TAG + DAG)	DAG	Pigments
GC	76.2% ± 6.5%	82.8%± 7.1%		
FT-IR	75.9% ± 22.1%	82.5% ± 24.1%		
TLC			10.7%	
HPLC				2.4% ± 0.8%
$scCO_2$	**Fatty Acids**	**Lipids**	**DAG**	**Pigments**
GC	83.2% ± 11.6%	87.3% ± 12.2%		
FT-IR	87.3% ± 17.3%	91.9% ± 18.2%		
TLC			0.0%	
HPLC				1.1% ± 0.3%

6 Ethenolysis

6.1 Ethenolysis of methyl oleate in dichloromethane

Ethenolysis experiments of methyl oleate in dichloromethane as a solvent were performed in a 20 mL pressure reactor with a glass inlay and a magnetic stirrer, heated in an aluminium block. The reactions were carried out under inert atmosphere. 2 mL (5.9 mmol) of methyl oleate was transferred via syringe into the reactor. Hoveyda-Grubbs 1st generation catalyst was weighed into a Schlenk tube and dissolved in 4 mL dichloromethane. This solution was added to the reactor. The reaction was stirred at ambient temperature and 10 bar ethylene for 6 h. The reactor was vented, the reaction mixture was quenched with ethyl vinyl ether and samples were analysed via GC.

Table S9 Ethenolysis of methyl oleate in dichloromethane.

Catalyst	Catalyst loading [mol%]	Conversion[a] [%]	Selectivity for ethenolysis products[a] [%]
G1	0.1	77	98
G1	0.5	87	98
HG1	0.1	75	97
HG1	0.5	95	96

G1: Grubbs 1st generation catalyst, HG1: Hoveyda-Grubbs 1st generation catalyst. Conditions: 2 mL (5.9 mmol, ca. 1 mol L^{-1}) methyl oleate, 10 bar ethylene (ca. 2 mol L^{-1} according to Ref 15, 4 mL CH_2Cl_2, ambient temperature, 6h. [a] Determined via GC analysis.

6.2 General procedure for ethenolysis in $scCO_2$

All experiments were carried out in the $scCO_2$ pressure reactor (Figure S1) at a constant reactor volume of 30 mL. In a glovebox the catalyst was weighed into a GC vial. The vial lid with the catalyst was placed on the stirrer blades in the high-pressure reactor. The fatty acid ester, the model substrate mixture or algae oil, respectively, was added via syringe, such that the catalyst and the starting material were not in contact at this point. After sealing of the reactor, CO_2 was added up to a total pressure of 200 bar and the reactor was heated. When the temperature reached 45 °C, CO_2 was added up to the final reaction pressure of 300 bar. Under these conditions, methyl oleate, the model substrate mixture and algae oil showed complete visible dissolution. After the desired time the reaction mixture was released into the expansion vessel containing a mixture of 80 mL CH_2Cl_2 and 20 mL ethyl vinyl ether. The reaction mixture was analyzed via GC.

6.3 Ethenolysis of methyl oleate in $scCO_2$

Table S10 Screening of conditions for ethenolysis of methyl oleate in $scCO_2$.

Catalyst	Catalyst loading [mol%]	Ethylene pressure [bar]	Reaction time [h]	Conversion[a] [%]	Selectivity[a] for ethenolysis products [%]
G1	0.1	10	6	50	95[b]
G1	1	10	6	61	>99
HG1	0.1	10	6	24	92[b]
HG1	0.1	10	18	26	>99
HG1	0.1	5	6	28	>99
HG1	0.2	10	6	65	>99
HG1	0.2	10	18	61	>99
HG1	0.2	5	6	56	>99
HG1	0.5	10	2	69	>99
HG1	0.5	10	6	88	>99
HG1	0.5	10	18	86	>99
HG1	0.5	5	6	83	>99
HG1	1	10	6	88	96[b]
HG1	1	10	18	88	97[b]

G1: Grubbs 1st generation catalyst, HG1: Hoveyda-Grubbs 1st generation catalyst. Conditions: 1.25 mL (3.7 mmol, ca. 0.1 mol L^{-1}) methyl oleate, total pressure of 300 bar at 45 °C, 30 mL reactor volume (10 bar ethylene = ca. 0.5 mol L^{-1}, 5 bar ethylene = ca. 0.3 mol L^{-1}. Based on an assumption of ca 2 mol L^{-1} saturation concentration in methyl oleate,[15] plus amount of ethylene in gas phase). [a] Determined via GC analysis. [b]The remainder are self-metathesis products.

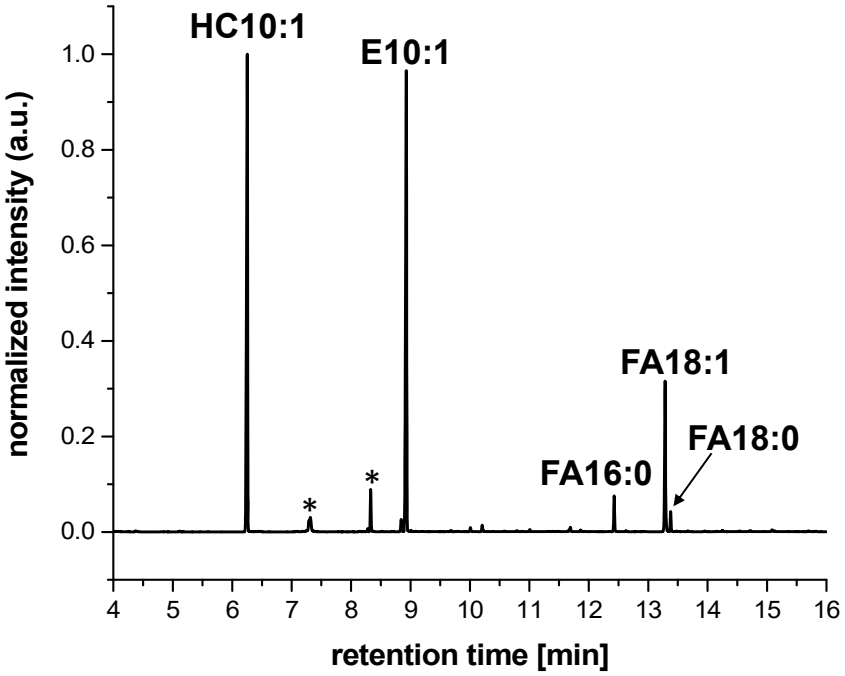


Figure S19 Gas chromatogram of the reaction mixture of ethenolysis in $scCO_2$ of methyl oleate with 0.5mol% Hoveyda-Grubbs 1st generation catalyst. The starting material (FA18:1 methyl oleate) and the ethenolysis products (E10:1 methyl 9-decenoate, HC10:1 1-decene) are labelled. Side-products likely originating from ethyl vinyl ether are labelled with an asterisk.

6.4 Ethenolysis of a model substrate mixture in $scCO_2$

A ratio of 4:5:1 (FA16:0 : FA18:1 : FA20:5) was chosen comparable to the ratio in the extracted algae oil. 2.11 g of the model substrate mixture (3.7 mmol double bonds), 10 bar ethylene and a catalyst loading of 0.5 mol% per double bond (Hoveyda-Grubbs 1st generation) were employed. The experiments were carried out in a constant reactor volume of 30 mL at a total pressure of 300 bar at 45 °C.

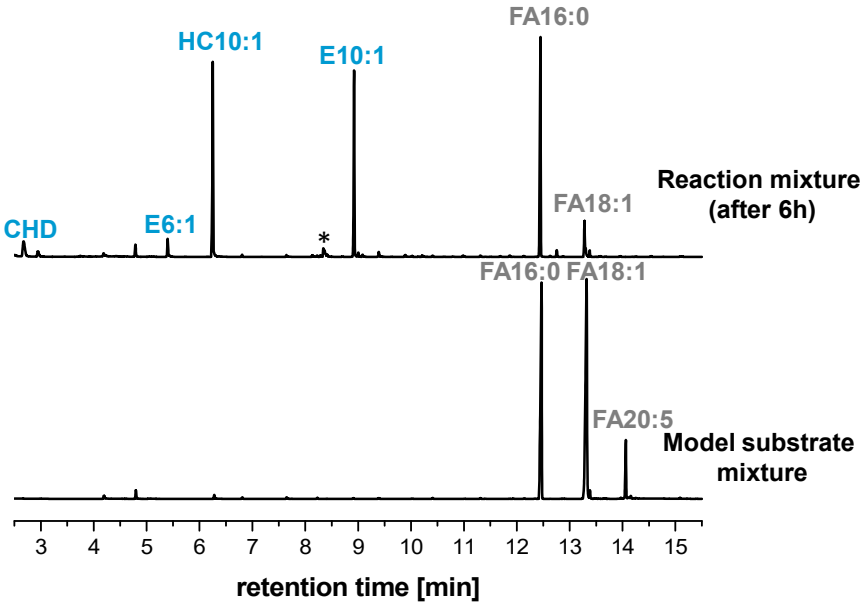


Figure S20 Gas chromatograms of the model substrate mixture (bottom) and the reaction mixture of the ethenolysis in $scCO_2$ of this model substrate mixture (top) with Hoveyda-Grubbs 1st generation catalyst and assignments of the products (CHD 1,4-cyclohexadiene, E6:1 methyl 5-hexenoate, HC10:1 1-decene, E10:1 methyl 9-decenoate) and the fatty acid esters (FA16:0 methyl palmitate, FA18:1 methyl oleate, FA20:5 methyl eicosapentaenoate). Side-products likely originating from ethyl vinyl ether are labelled with an asterisk.

6.5 Ethenolysis of $scCO_2$ extracted algae oil in $scCO_2$

0.9 g $scCO_2$ extracted algae oil, which corresponds to 3.7 mmol double bonds, 10 bar ethylene and a catalyst loading of 0.5 mol% per double bond (Hoveyda-Grubbs 1st generation) were used. The experiments were carried out at a total pressure of 300 bar at 45 °C in a constant reactor volume of 30 mL. After 6 hours, the contents of the reactor were vented into the expansion vessel, bubbling the gas stream through a mixture of 80 mL CH_2Cl_2 and 20 mL ethyl vinyl ether. The resulting solution of the reaction mixture was analyzed via GC. For this purpose, a GC sample was esterified with methanol and a catalytic amount of sulfuric acid at 50 °C for 3 days.

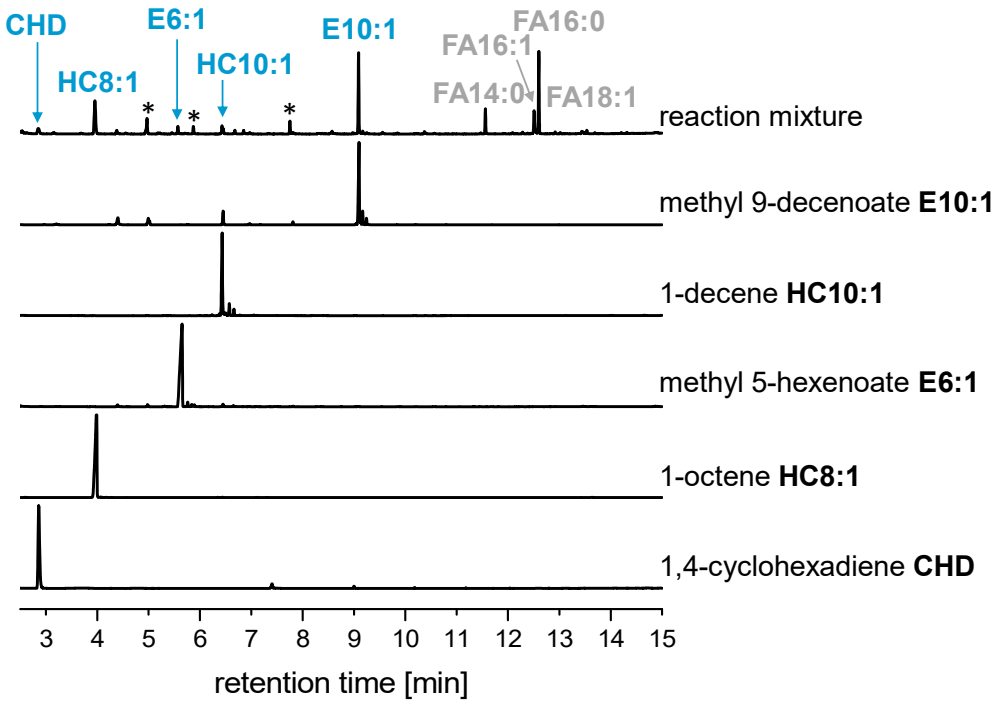


Figure S21 Gas chromatogram of the ethenolysis of $scCO_2$ extracted algae oil in $scCO_2$ (after transesterification with methanol) with Hoveyda-Grubbs 1st generation catalyst (top) and gas chromatograms of genuine samples of the ethenolysis products and the self-metathesis product 1,4-cyclohexadiene CHD for identification. Side-products likely originating from ethyl vinyl ether are labelled with an asterisk.

6.6 Simultaneous extraction and ethenolysis

The wet algae were pre-treated with ultrasound (for 10 min with a pulse of 10 s and amplitude of 60%), freeze-dried and crushed with a pestle and mortal. 1 g of these pre-treated and freeze-dried algae were placed into the reactor. This amount corresponds approximately to 0.25 g algae oil and 1 mmol double bonds. In the glovebox the catalyst (0.5 mol% Hoveyda-Grubbs 1st generation per double bond) was weighed into a vial cap and transferred to the high-pressure reactor with a constant reactor volume of 30 mL. The catalyst and the starting material were not in contact at this point. After sealing of the reactor, 10 bar ethylene and subsequently CO_2 was added up to a total pressure of 200 bar and the reactor was heated. When the temperature reached 45 °C, CO_2 was added up to final reaction pressure of 300 bar. After 18 hours, the contents of the reactor were vented into the expansion vessel bubbling the gas stream through a mixture of 80 mL CH_2Cl_2 and 20 mL ethyl vinyl ether. The reaction mixture was analyzed via GC. For this purpose, a GC sample was esterified with methanol and a catalytic amount of sulfuric acid at 50°C for 3 days.

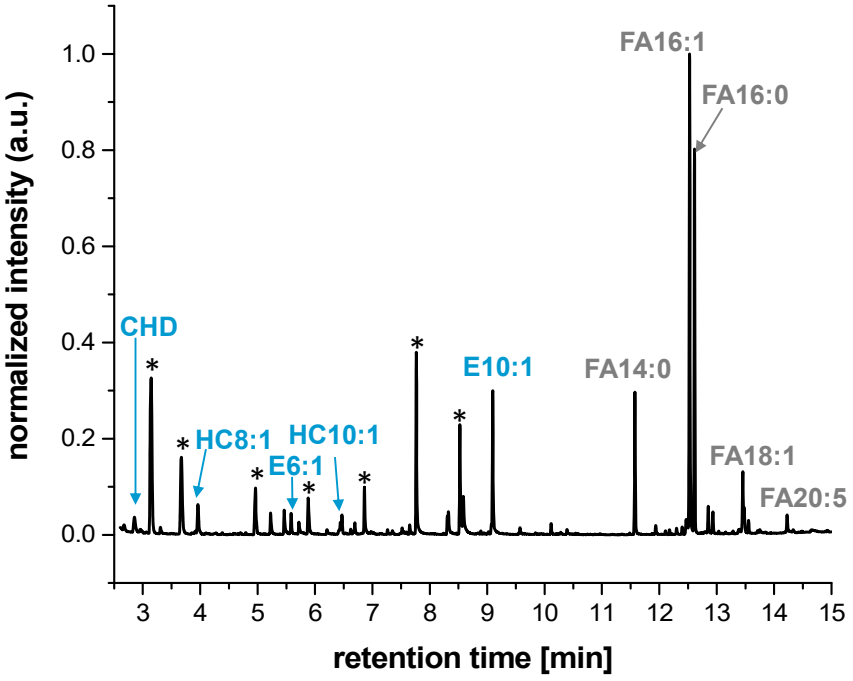


Figure S22 Gas chromatogram (after transesterification for GC analysis) of ethenolysis in $scCO_2$ of ultrasound pre-treated freeze-dried algae with Hoveyda-Grubbs 1st generation catalyst and assignments of the ethenolysis products (HC8:1 1-octene, E6:1 methyl 5-hexenoate, HC10:1 1-decene, E10:1 methyl 9-decenoate), the self-metathesis product CHD 1,4-cyclohexadiene and the fatty acid esters (FA14:0 methyl myristate, FA16:1 methyl palmitoleate, FA16:0 methyl palmitate, FA18:1 methyl oleate, FA20:5 methyl eicosapentaenoate). Side-products likely originating from ethyl vinyl ether are labelled with an asterisk.

7 Butenolysis in $scCO_2$

7.1 General procedure for butenolysis in $scCO_2$

In the glovebox Hoveyda-Grubbs 2nd generation catalyst was weighed into a GC vial. In case of 30 mL reactor volume the lid, or with 60 mL volume the vial with the catalyst, was placed on the stirrer blades in the high-pressure reactor. The fatty acid ester, the model substrate mixture, or algae oil, respectively, was added via syringe, such that the catalyst and the starting material were not in contact at this point. Subsequently, 2-butene (10-fold excess) was added with a cooled syringe and the reactor was closed immediately. CO_2 was added up to a total pressure of 200 bar and the reactor was heated. When the temperature reached 45 °C, CO_2 was added up to the final reaction pressure of 300 bar. Under these conditions, methyl oleate, the model substrate mixture and algae oil showed complete visible dissolution. After 2 hours, the contents of the reactor were vented into the expansion vessel, bubbling the

gas stream through a mixture of 80 mL CH_2Cl_2 and 20 mL ethyl vinyl ether. The reaction mixture was analyzed via GC.

7.2 Butenolysis of methyl oleate in $scCO_2$

Table S11 Conversions and selectivities of butenolysis of methyl oleate with different catalyst loadings in $scCO_2$.

Catalyst loading [mol%]	Conversion[a] [%]	Selectivity[a] for butenolysis products [%]
0.01	25	91
0.05	82	95
0.10	93	94

Conditions: Hoveyda-Grubbs 2nd generation, 7.37 mmol methyl oleate, ten-fold excess of 2-butene, 300 bar CO_2 at 45 °C, constant reactor volume of 60 mL, 2 h. [a] Determined via GC analysis.

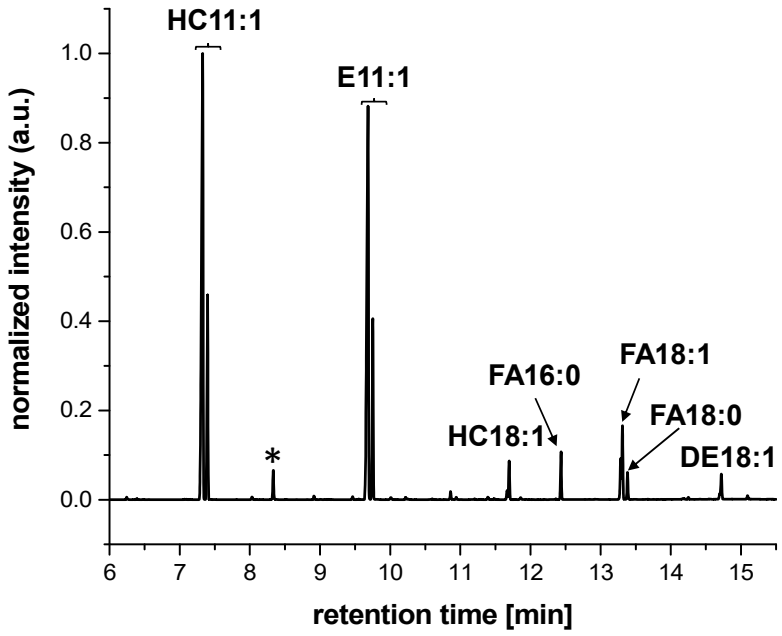


Figure S23 Gas chromatogram of the reaction mixture of butenolysis in $scCO_2$ of methyl oleate with 0.1 mol% Hoveyda-Grubbs 2nd generation catalyst. The starting material (FA18:1 methyl oleate), the butenolysis products (E11:1 methyl 9-undecenoate, HC11:1 2-undecene, both *trans* and *cis* isomers in a ratio of 80:20) and self-metathesis products (HC18:1 9-octadecene, DE18:1 dimethyl 9-octadecenedioate) are labelled. Side-products likely originating from ethyl vinyl ether are labelled with an asterisk.

7.3 Butenolysis of a model substrate mixture in $scCO_2$

A ratio of 4:5:1 (FA16:0 : FA18:1 : FA20:5) was chosen comparable to the ratio in the extracted algae oil. 2.11 g of the model mixture (3.7 mmol double bonds), 73.7 mmol 2-butene and a catalyst loading of 0.1 mol% per double bond (Hoveyda-Grubbs 2nd generation) were used. The experiments were carried out at a constant reactor volume of 30 mL.

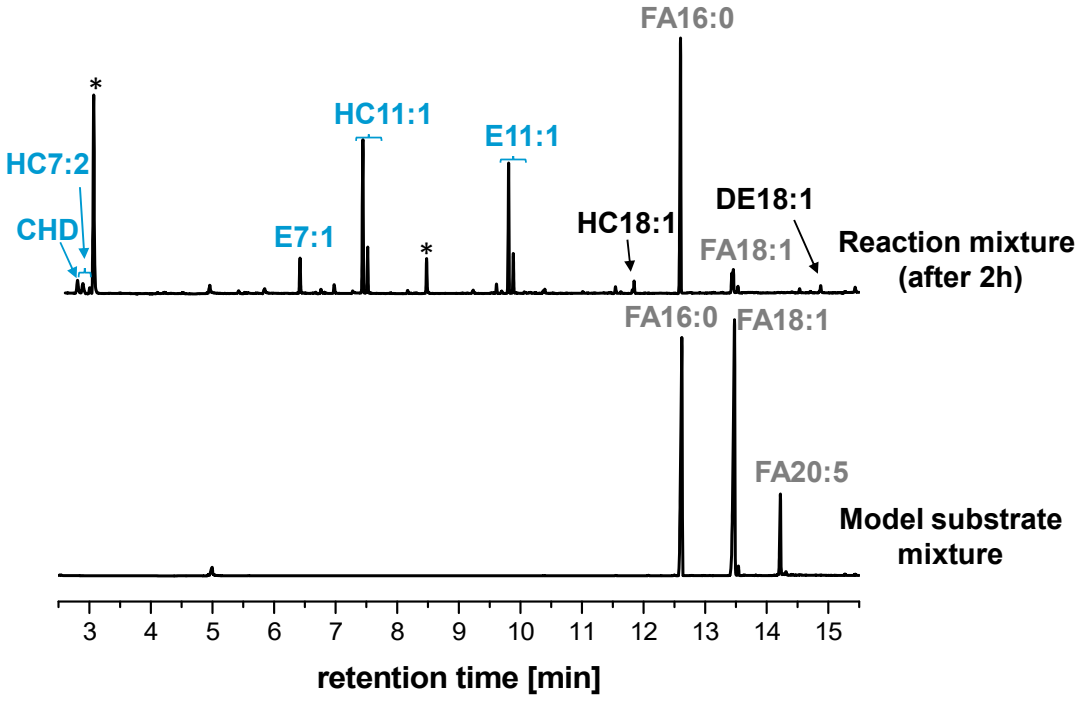


Figure S24 Gas chromatograms of a model substrate mixture (bottom) and the reaction mixture of the butenolysis in $scCO_2$ of this model substrate mixture (top) with Hoveyda-Grubbs 2nd generation catalyst and assignments of the butenolysis products (HC7:2 2,5-heptadiene, E7:1 methyl 5-heptenoate, HC11:1 2-undecene, E11:1 methyl 9-undecenoate), self-metathesis products (CHD 1,4-cyclohexadiene, HC18:1 9-octadecene, DE18:1 dimethyl 9-octadecenedioate) and the fatty acid esters (FA16:0 methyl palmitate, FA18:1 methyl oleate, FA20:5 methyl eicosapentaenoate). Side-products likely originating from ethyl vinyl ether are labelled with an asterisk.

7.4 Butenolysis of $scCO_2$ extracted algae oil in $scCO_2$

0.9 g $scCO_2$ extracted algae oil, which corresponds to 3,7 mmol double bonds, 37 mmol 2-butene and a catalyst loading of 0.1 mol% per double bond (Hoveyda-Grubbs 2nd generation) were used. The experiments were carried out at a constant reactor volume of 30 mL. For GC analysis, a sample was esterified with methanol and catalytic amount of sulfuric acid at 50 °C for 3 days.

7.5 Simultaneous extraction and butenolysis

The wet algae were pre-treated with ultrasound (for 10 min with a pulse of 10 s and amplitude of 60%), freeze-dried, and crushed with a pestle and mortal. 1 g of these pre-treated and freeze-dried algae were placed into the reactor. This amount corresponds approximately to 0.25 g algae oil and 1 mmol double bonds. In the glovebox the catalyst (0.1 mol% Hoveyda-Grubbs 2nd generation per double bond) was weighed into a vial cap and transferred to the high-pressure reactor with a constant reactor volume of 30 mL. The catalyst and the starting material were not in contact at this point. 0.9 mL 2-butene (10-fold excess per double bond) was added with a cooled syringe and the reactor was closed immediately. CO_2 was added up to a total pressure of 200 bar and the reactor was heated. When the temperature reached 45 °C, CO_2 was added up to the final reaction pressure of 300 bar. After 3 hours, the contents of the reactor were vented into the expansion vessel bubbling the gas stream through a mixture of 80 mL CH_2Cl_2 and 20 mL ethyl vinyl ether. For GC analysis, a sample was esterified with methanol and a catalytic amount of sulfuric acid at 50 °C for 3 days.

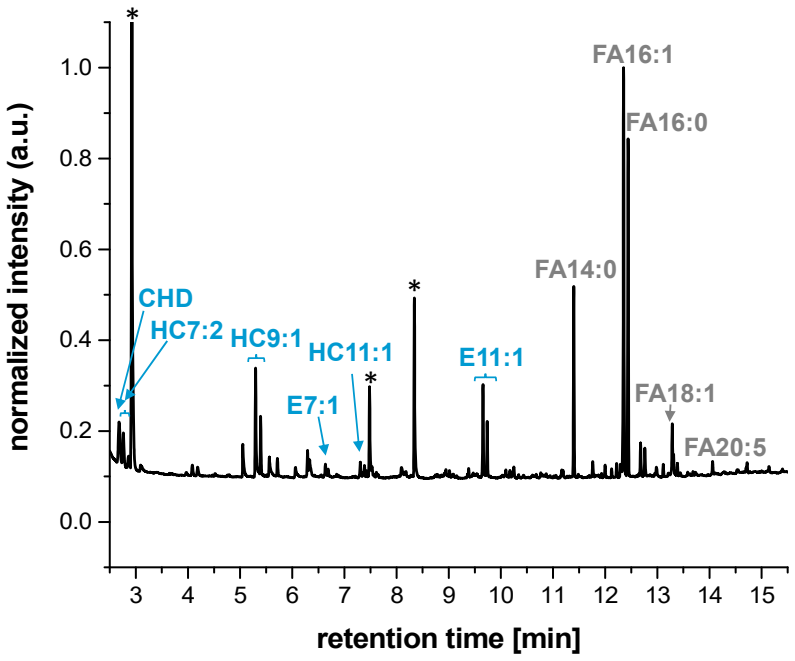


Figure S25 Gas chromatogram (after transesterification for GC analysis) of butenolysis in scCO_2 of ultrasound pre-treated freeze-dried algae with Hoveyda-Grubbs 2nd generation catalyst and assignments of the butenolysis products (HC7:2 2,5-heptadiene, HC9:1 2-nonene, E7:1 methyl 5-heptenoate, HC11:1 2-undecene, E11:1 methyl 9-undecenoate), the self-metathesis product CHD 1,4-cyclohexadiene and the fatty acid esters (FA14:0 methyl myristate, FA16:1 methyl palmitoleate, FA16:0 methyl palmitate, FA18:1 methyl oleate). Side-products likely originating from ethyl vinyl ether are labelled with an asterisk.

8 Isomerizing alkoxycarbonylation in $scCO_2$

8.1 General procedure of isomerizing alkoxycarbonylation in $scCO_2$

In a glove-box, catalyst precursor [Pd(OTf)(dtbpx)]OTf (37.7 mg, 0.0472 mmol) was placed in a Schlenk tube. The Schlenk tube was transferred out of the glove-box and half of the required MeOH was added (14 mL unless indicated otherwise). In a separate Schlenk tube, methyl oleate 5.9 mmol or extracted algae oil 1-1.3 g, respectively was placed. The remaining amount of the MeOH (14 mL) was added followed by protonated diphosphine ligand (dtbpx $2H^+$, 5 equiv. 0.236 mmol). Both mixtures were transferred to the high-pressure reactor (60 mL volume). The reactor was closed, CO was added (30 bar) and heated to 90 °C followed by the addition of CO_2 up to 325 bar. The reactor volume was kept constant at 60 mL and it was stirred at 325 bar and 90 °C for the indicated time. The contents of the reactor were vented into the expansion vessel and the material was washed out with CH_2Cl_2. After collection, the solvent was evaporated, and the mixture was analysed by GC.

8.2 Simultaneous extraction and isomerizing alkoxycarbonylation

In a glove-box, catalyst precursor [Pd(dtbpx)$(OTf)_2$] (37.7 mg, 0.0472 mmol) was weighed into a Schlenk tube and was dissolved in 14 mL MeOH. Freeze-dried algae (1 g) were placed in a separate Schlenk tube and 14 mL MeOH and 0.236 mmol dtbpx $2H^+$ were added. Both mixtures were transferred to the high-pressure reactor. The reactor was closed, CO was added (30 bar) and heated to 90 °C followed by the addition of CO_2 up to 325 bar. The reactor volume was kept constant and it was stirred at 325 bar and 90 °C for the indicated time. The contents of the reactor were released into the expansion vessel and the material was washed out with CH_2Cl_2. After collection, the solvent was evaporated, and the mixture was analysed by GC.

Table S12 Alkoxycarbonylation of methyl oleate (MO), $scCO_2$ extracted algae oil and freeze-dried algae in dense CO_2.

Entry	Substrate (amount)	Reaction time (h)	Conversion (%)[a]	Linear selectivity (%)[b]
1	MO(6 mmol)	18	29	92
2	Algae oil (1 g)	96	50	89
3	Freeze-dried algae (1 g)	96	90	93

Conditions: $Pd(dtbpx)(OTf)_2$ (0.0472 mmol, 37.7 mg), $[dtbpxH^+]_2OTf^-$ (0.236 mmol, 163.8 mg), 28 mL MeOH, 30 bar CO, total pressure of 325 bar at 90 °C. The reactor volume was kept constant at 60 mL. All mixtures were analysed by gas chromatography. [a]Calculated as conversion of 16:1 and 18:1. [b]Ratio of linear diesters vs. all diesters.

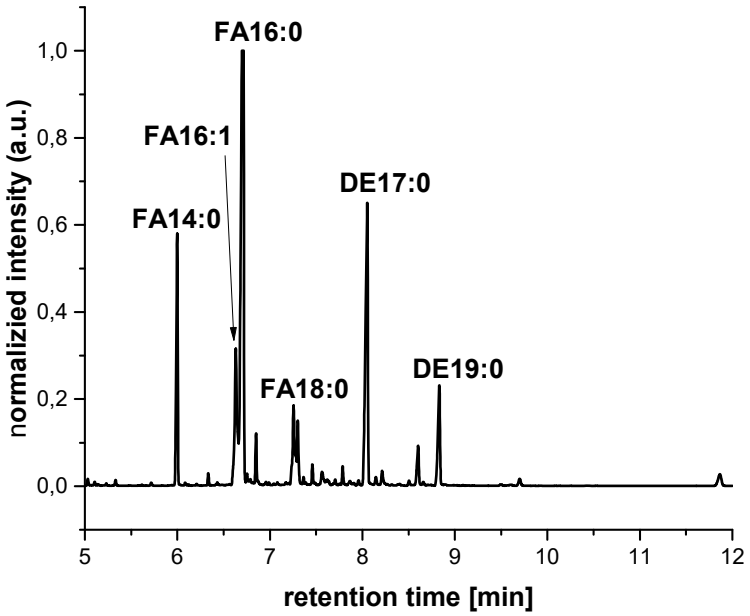


Figure S26 Typical gas chromatogram of a post-alkoxycarbonylation mixture of crude freeze-dried algae (*P. tricornutum*).

9 Further gas chromatographic data

9.1 Additional gas chromatographic signals from quenching with ethyl vinyl ether

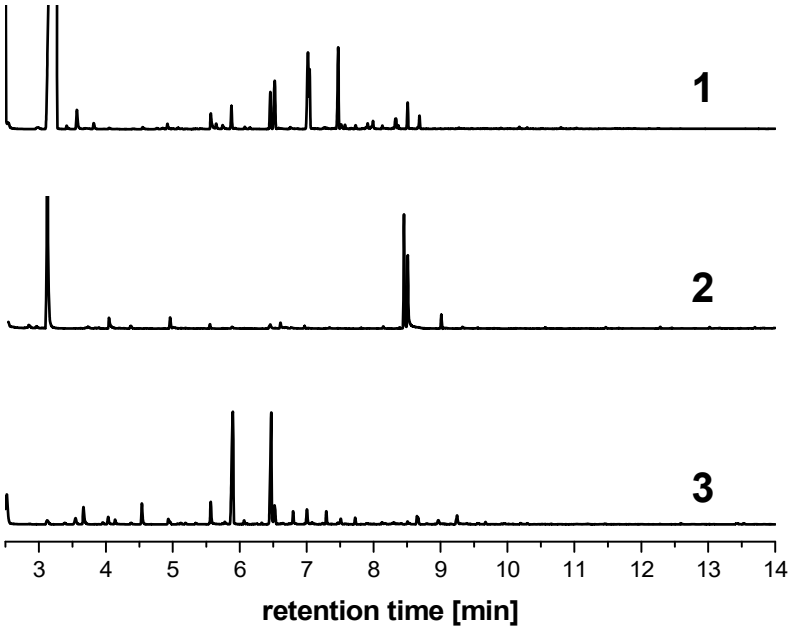


Figure S27 1: Gas chromatogram of Hoveyda-Grubbs 1st generation catalyst in methanol, dichloromethane and a catalytic amount of sulfuric acid quenched with ethyl vinyl ether. 2: Gas chromatogram of Hoveyda-Grubbs 2nd generation catalyst in methanol, dichloromethane and a catalytic amount of sulfuric acid quenched with ethyl vinyl ether. 3: Gas chromatogram of ethyl vinyl ether in methanol, dichloromethane and a catalytic amount of sulfuric acid heated to 50 °C for 3 days.

9.2 Abbreviations and response factors

All response factors were determined as an average of three measurements against dodecane.

Table S13 Substances and their abbreviation with the corresponding response factors.

Name	Abbreviation	Response factor (mol/mol)	Response factor (w/w)
Methyl myristate	FA14:0	0.93	0.70
Methyl palmitoleate	FA16:1	1.09	0.69
Methyl palmitate	FA16:0	1.03	0.68
Methyl oleate	FA18:1	1.30	0.75
Methyl stearate	FA18:0	1.13	0.64
Methyl eicosapentaenoate	FA20:5	0.79	0.45
1,4-Cyclohexadiene	CHD	0.41	0.86
Methyl 5-hexenoate	E6:1	0.42*	0.56*
1-Octene	HC8:1	0.65	0.98
1-Decene	HC10:1	0.80*	0.97*
Methyl 9-decenoate	E10:1	0.74*	0.68*
Methyl 5-heptenoate	E7:1	0.47*	0.56*
2,5-Heptadiene	HC7:2	0.54	0.94
Methyl 9-undecenoate	E11:1	0.76	0.70
2-Nonene	HC9:1	0.75*	1.01*
2-Undecene	HC11:1	0.90*	0.99*
1,17-diester	DE17:0	1.05	0.54
1,19-diester	DE19:0	1.25	0.59

*Derived from comparable compounds.

10 Complete data for Tables 1 to 3

Table S14 Ethenolysis of unsaturated fatty acids of a model substrate mixture and $scCO_2$ extracted algae oil with conversions of the components and selectivities to ethenolysis products and self-metathesis product 1,4-cyclohexadiene (CHD).

Ethenolysis of		model substrate mixture[a]		$scCO_2$ extracted algae oil	
		1	**2**	**1**	**2**
Composition of the initial reaction mixture [%]	**FA14:0**	-	-	9	9
	FA16:1	-	-	49	49
	FA16:0	40	40	27	27
	FA18:1	50	50	4	4
	FA18:0	-	-	2	2
	FA20:5	10	10	9	9
Conversion [%][b]	**FA16:1**	-		83	79
	FA18:1	85	86	88	92
	FA20:5	>99	>99	94	89
Selectivity [%][c] for	**CHD**	82	86	61	68
	E6:1	83	87	84	79
	HC8:1	-	-	79	86
	HC10:1	>99	>99	>99	94
	E10:1	>99	>99	85	83

Conditions: 0.5 mol% Hoveyda-Grubbs 1st generation catalyst per double bond, 10 bar ethylene, 300 bar CO_2 (total pressure) at 45°C, 6 h. [a]Mixture of 40% FA16:0, 50% FA18:1, 10% FA20:5, [b]determined over FA16:0 as an internal standard [c] the selectivity to a product is defined as the ratio of the product to the theoretical maximum amount of this product at complete ethenolysis or in case of CHD (1,4-cyclohexadiene) complete self-metathesis of FA20:5 (determined over FA16:0 as an internal standard) in the gas chromatogram.

Table S15 Butenolysis of unsaturated fatty acids of a model substrate mixture and $scCO_2$ extracted algae oil with conversions of the components and selectivities for butenolysis products and self-metathesis product 1,4-cyclohexadiene (CHD).

Butenolysis of		**model substrate mixture[a]**		**$scCO_2$ extracted algae oil**	
		1	2	1	2
Composition of the initial reaction mixture [%]	**FA14:0**	-	-	9	9
	FA16:1	-	-	47	47
	FA16:0	40	40	25	25
	FA18:1	50	50	8	8
	FA18:0	-	-	1	1
	FA20:5	10	10	10	10
Conversion [%][b]	**FA16:1**	-	-	79	83
	FA18:1	88	88	93	88
	FA20:5	>99	95	>99	>99
Selectivity [%][c] for	**CHD**	25	29	26	22
	HC7:2	55	49	57	54
	E7:1	65	68	67	63
	HC9:1	-	-	88	82
	HC11:1	88	81	84	82
	E11:1	88	87	80	84

Conditions: 0.1 mol% Hoveyda-Grubbs 2nd generation catalyst, 10-fold excess of 2-butene, 300 bar CO_2 (total pressure) at 45°C, 2 h. [a]Mixture of 40% FA16:0, 50% FA18:1, 10% FA20:5, [b]determined via FA16:0 as an internal standard, [c] the selectivity to a product is defined as the ratio of the product to the theoretical maximum amount of this product at complete butenolysis or in case of CHD (1,4-cyclohexadiene) complete self-metathesis of FA20:5 (determined via FA16:0 as an internal standard) in the gas chromatograms.

Table S16 Integrated procedure of extraction and cross-metathesis of freeze-dried algae in $scCO_2$ with selectivities and conversions of the unsaturated fatty acids.

	Combined extraction and ethenolysis[a] of freeze-dried algae			Combined extraction and butenolysis[b] of freeze-dried algae		
		1	**2**		**1**	**2**
Conversion [%][c]	**FA16:1**	31	34	**FA16:1**	44	50
	FA18:1	46	41	**FA18:1**	65	64
	FA20:5	86	90	**FA20:5**	91	87
Selectivity [%][d] for	**CHD**	46	53	**CHD**	34	37
	HC5:2[e]	-	-	**HC7:2**	67	72
	E6:1	92	83	**E7:1**	64	71
	HC8:1	63	56	**HC9:1**	91	>99
	HC10:1	88	88	**HC11:1**	>99	95
	E10:1	78	76	**E11:1**	72	67

The fresh algae were ultrasonicated and freeze-dried. The composition of fatty acids in the freeze-dried algae were assumed to be the same as for the $scCO_2$ extracted algae oil. The reaction mixture was analyzed via gas chromatography after transesterification and filtration. [a]Conditions: 0.5 mol% Hoveyda-Grubbs 1st generation catalyst, 10 bar ethylene, 300 bar CO_2 (total pressure) at 45°C, 18 h. [b]Conditions: 0.1 mol% Hoveyda-Grubbs 2nd generation catalyst, 10 fold-excess of 2-butene, 300 bar CO_2 (total pressure) at 45°C, 3 h. [c]Conversions were determined via gas chromatography via the FA16:0 present in the algae oil as an internal standard. [d]The selectivity for a product is defined as the ratio of the product to the theoretical maximum amount of this product at complete ethenolysis or butenolysis or in case of CHD (1,4-cyclohexadiene) complete self-metathesis of FA20:5 (determined over FA16:0 as an internal standard) in GC. [e]Not detectable via GC due to its low boiling point.

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