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

Julia Zimmerer, Dennis Pingen, Sandra K. Hess, Tobias Koengeter and Stefan Mecking *

Fatty acids from microalgae are attractive compounds for catalytic upgrading to chemicals, but their extraction often requires multi-step procedures and the use of various organic solvents. To relieve this bottleneck, we propose a straightforward approach of combined extraction and catalytic functionalization *via* olefin cross-metathesis (ethenolysis and butenolysis) in supercritical CO_2 ($scCO_2$). This is demonstrated for *Phaeodactylum tricornutum* microalgae biomass. $ScCO_2$ at optimum conditions (90 °C, 620 atm, $\rho(CO_2)$ = 0.90 g mL^{-1}) extracted the lipids selectively and quantitatively from previously disrupted cells, while organic solvent extraction for comparison additionally extracted polar diacylglycerides and chlorophylls. In a one-pot approach, olefin cross-metathesis of the unsaturated fatty acids (FA16:1, FA18:1 and FA20:5) by alkenolysis yielded the desirable mid-chain olefin and unsaturated ester products. The product spectrum compares to alkenolysis of individual model compounds in $scCO_2$ as well as of separately $scCO_2$ extracted microalgae oil. Both these ethenolysis and butenolysis proceed with conversions of more than 81% and high selectivities to the desired products. This biorefinery approach was further illustrated by the simultaneous extraction and catalytic isomerizing alkoxycarbonylation in $scCO_2$.

Introduction

Microalgae are a promising future feedstock. Unlike traditional crops like soy, palms or sunflowers they do not require arable land. Also, per acre yields can be much higher. Microalgae can be cultivated in fresh, salt or brackish water.[1] Autotrophically or heterotrophically grown microalgae are an established source of high value food additives and pigments. Further, the production of fuels from microalgae oils has been demonstrated on a multiton scale. By comparison to fuels, a production of higher value chemicals would appear more sensible.[2,3] Unlike fuel production, which downgrades the plant oil feedstock to hydrocarbons, the production of chemicals can take advantage of the particular molecular structure of the feedstock. Notably, microalgae oils can contain unique fatty acids with unusual chain lengths and multiple double bonds not found in traditional plant oils.[1,4,5]

Yet the usage of biomass has a significant bottleneck; the isolation of the desired substrates and extraction often requires energy-intensive multi-step procedures and the use of environmentally harmful organic solvents. This also applies to microalgae, where the achievable cell densities are limited. To address this problem, we pursue an integration of the biomass extraction with the catalytic upgrading in a common solvent, supercritical carbon dioxide ($scCO_2$. Fig. 1).

Advantageously, $scCO_2$ is capable of the selective extraction of lipids from microalgae[6–11] and various catalytic reactions of interest have been reported to be compatible with $scCO_2$ as a solvent.[12–16] Additionally, $scCO_2$ is non-toxic, non-flammable and it can be easily removed. For the upgrading of palm oil, alkenolysis is an established reaction.[17] Considering microalgae oils as a feedstock, alkenolysis of their unconventional

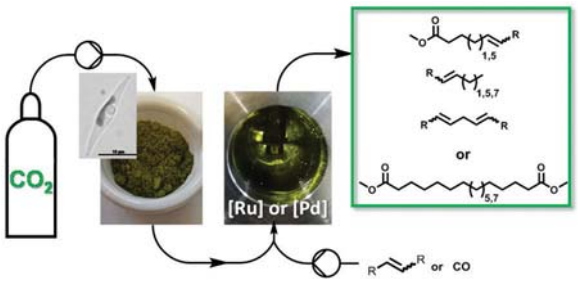


Fig. 1 Schematic process design of extraction and catalytic valorization of microalgae in $scCO_2$ to mid-chain olefins, unsaturated esters and long-chain α,ω-diesters.

Chair of Chemical Materials Science, Department of Chemistry, University of Konstanz, 78464 Konstanz, Germany.
E-mail: stefan.mecking@uni-konstanz.de; Fax: +49 7531 88-5152; Tel: +49 7531 88-5151

fatty acids (*vide supra*) can provide a range of useful compounds, difficult to access otherwise.[18] In literature ruthenium-based olefin metathesis has been reported to be compatible with $scCO_2$.[19–21]

Results and discussion

Extraction and characterization of algae oil from *Phaeodactylum tricornutum*

We employed *Phaeodactylum tricornutum* for our studies, as this algae strain is robust and can contain high amounts of unsaturated fatty acids.[22] To find suitable extraction conditions, 1 g of freeze-dried algae (harvested in the late stationary phase) was subjected to $scCO_2$ extraction at different pressures and temperatures, and thus also different densities[23] (Table S2 and Fig. S6 in the ESI†).

At a given density, the efficiency of extraction as reflected by the yield of algae oil increases with increasing extraction temperatures. The extraction pressure is often the technically limiting factor, given by the equipment employed. Notably, for a given pressure, the yield increases with increasing temperature, even though the $scCO_2$ density decreases. Disruption of the microalgae cells by ultrasonication[24,25] (Fig. S4†) prior to extraction enhanced yields by *ca.* 20% to 30%. Under the optimum conditions of the range investigated, 90 °C and 621 bar corresponding to ρ = 0.9 g mL^{-1}, 25 wt% yield of algae oil were obtained (Fig. 2).

As a reference, an algae sample was extracted with organic solvent using a mixture of water, methanol and chloroform (3 : 4 : 8), as described by Folch.[26] This method, which quantitatively captures all fatty acid compounds present in the microalgae, yielded 28 wt% of extracted algae oil.

Gas chromatographic (GC) analysis of the extracted oils reveals similar fatty acid compositions, largely independent of the $scCO_2$ extraction conditions (Fig. 3). With only small variations (*ca.* 2%), 55% of mono-unsaturated fatty acids (47% palmitoleic acid (**FA16:1**) and 8% oleic acid (**FA18:1**)), 10% of the multiple unsaturated fatty acid eicosapentaenoic acid (**FA20:5**), and 35% of saturated fatty acids (<1% stearic acid (**FA18:0**), 25% palmitic acid (**FA16:0**) and 9% myristic acid (**FA14:0**)) are present. In total the algae oil extracted by means of $scCO_2$ contains around 4 mmol of double bonds per 1 gram of oil, as quantified *via* an internal GC standard. By comparison, the oil extracted *via* the modified Folch method has a similar fatty acid profile (see Fig. S5†).

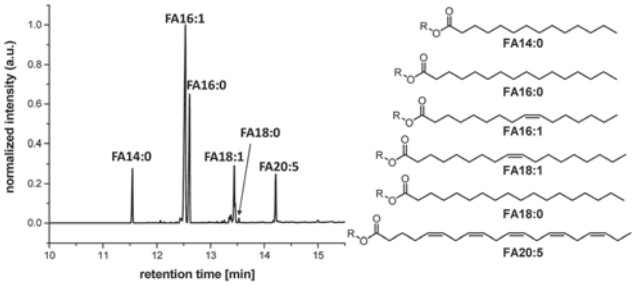


Fig. 3 Gas chromatogram of $scCO_2$ extracted algae oil from *Phaeodactylum tricornutum* (after transesterification with methanol for GC analysis). Myristic acid methyl ester FA14:0 9%, palmitoleic acid methyl ester FA16:1 47%, palmitic acid methyl ester FA16:0 25%, oleic acid methyl ester FA18:1 8%, stearic acid methyl ester FA18:0 <1%, eicosapentaenoic acid methyl ester FA20:5 10%.

The entire composition of the extracted oils in terms of classes of compounds present was quantified by a comprehensive analysis by a combination of methods (*cf.* ESI†). The lipid composition was analyzed *via* thin-layer chromatography (TLC),[27,28] pigments were quantified *via* high-performance liquid chromatography (HPLC)[29] and the content of proteins and carbohydrates was determined *via* Fourier-transform infrared spectroscopy (FT-IR).[30] Polar diacylglycerides[31] and chlorophylls[32] were found to not be extracted by the apolar $scCO_2$ (Fig. S14 and S17†), in line with previous findings.[33] Proteins were also not detected in the investigated oils (Fig. S14†), corresponding to a protein content below 2% (detection limit of FT-IR). Compared to the aforementioned Folch organic solvent extraction, extraction with $scCO_2$ as a nonpolar solvent is indeed more selective for the desired triacylglycerides, and carotenoids. This is also reflected by the color of the samples (Fig. S7†). The amount of pigments and polar diacylglycerides, which could interfere with catalysts, are 1.1% and below the detection limit (<5 wt% for TLC), respectively, for $scCO_2$ extracted oil. By comparison, with Folch's organic solvent mixture also substantial amounts (~10%) of phospho- and glycolipids (Fig. S11 and S12†) as well as chlorophylls (Fig. S17 and S18†) are extracted from the microalgae samples (Table S8†). This accounts for the differences in yields, that is, $scCO_2$ extraction under the conditions studied quantitatively extracts the triacylglycerides from the microalgae biomass.

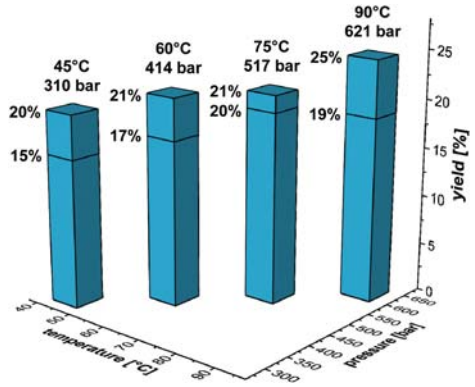
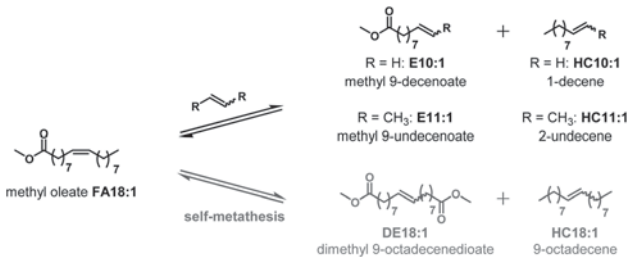


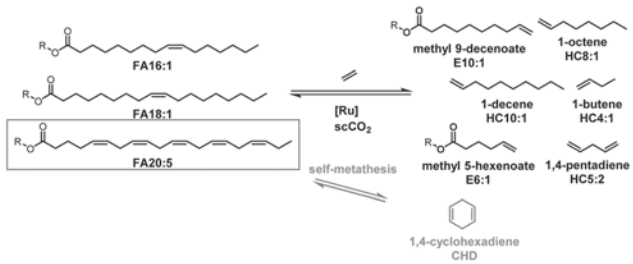
Fig. 2 Yields of $scCO_2$ extraction at different pressures and temperatures at constant density (0.9 g mL^{-1}). Lower part of the bar: extraction of freeze-dried algae, lower and upper part of the bar: extraction of ultrasound pre-treated freeze-dried algae.

Ethenolysis of model compounds and microalgae oil

Catalytic upgrading of fatty acids from microalgae *via* cross-metathesis gives access to a broad spectrum of unsaturated



Scheme 1 Self- and cross-metathesis of methyl oleate with ethylene or 2-butene.



Scheme 2 Ethenolysis products of the unsaturated fatty acids present in algae oil from *P. tricornutum,* and CHD as a self-metathesis product of FA20:5.

compounds. A general scheme of cross-metathesis of methyl oleate with ethylene or 2-butene is shown in Scheme 1.

The ethenolysis of unsaturated fatty acids in $scCO_2$ as reaction medium was first investigated with methyl oleate as a model compound which gives methyl 9-decenoate **E10:1** and 1-decene **HC10:1** as the desired products (Scheme 1, R = H).

Based on the results of the $scCO_2$ extraction of algae oil and with respect to the limited thermal stability of the metathesis catalyst, a temperature of 45 °C and a pressure of 300 bar were chosen for all ethenolysis experiments. In accordance to Song *et al.*,[34] 1 mol% of Grubbs 1st generation catalyst was employed. With 10 bar ethylene and total pressure of 300 bar at 45 °C a conversion of 61% of methyl oleate was achieved (Table S10†). No self-metathesis products (<1%) of methyl oleate (dimethyl 9-octadecendioate **DE18:1** and 9-octadecene **HC18:1**, Scheme 1) were observed. Due to promising results with Hoveyda–Grubbs 1st generation catalyst in the ethenolysis of methyl oleate in dichloromethane (Table S9†), this catalyst was also applied in the ethenolysis with $scCO_2$ as solvent. Under the same conditions as for Grubbs 1st generation catalyst (1 mol% catalyst, 10 bar ethylene, 300 bar total pressure at 45 °C), Hoveyda–Grubbs 1st generation catalyst gave a higher conversion of 88% and again almost no self-metathesis products were observed. Based on these results, the ethenolysis in $scCO_2$ was further investigated with Hoveyda–Grubbs 1st generation catalyst. The highest conversions of up to 88% were obtained using a catalyst loading of 0.5 mol% or 1 mol%, respectively, at an ethylene pressure of 10 bar. Conversions could not be improved neither by lowering the ethylene pressure nor by increasing the reaction time (see Table S10†).

Considering an effect on the solubility of the catalyst precursor, Grubbs 1st and 2nd generation catalysts have been suggested to be insoluble in $scCO_2$ (40 °C, 140 bar corresponds to 0.75 g mL^{-1}).[20] While we cannot exclude that under our conditions a dissolution of the catalyst precursor (Hoveyda–Grubbs 2nd generation catalyst, Grubbs 1st and 2nd generation catalyst, respectively) is assisted by reaction with the fatty acid substrate, a comparison of conversion in ethenolysis in $scCO_2$ *vs.* dichloromethane as a solvent (Tables S10 and S9†) suggests that in $scCO_2$ catalyst solubility is not an issue and a substantial portion or the entire amount of catalyst precursor is dissolved.

The ethenolysis of algae lipid feedstocks will provide a more complex spectrum of unsaturated products (Scheme 2): from **FA16:1** and **FA18:1** 1-octene **HC8:1**, 1-decene **HC10:1** and methyl 9-decenoate **E10:1** is formed, whereas complete ethenolysis of **FA20:5** results in 1,4-pentadiene **HC5:2**, methyl 5-hexenoate **E6:1** and 1-butene **HC4:1.** Yet, 1,4-pentadiene **HC5:2** and 1-butene **HC4:1** could not be quantified by GC analysis due to their low boiling point. Please note, complete ethenolysis of **FA20:5** can generate up to four equivalents of **HC5:2.** Furthermore, instead of cross-metathesis, up to two equivalents of 1,4-cyclohexadiene **CHD** can be generated in a self-metathesis reaction of **FA20:5**. Its intramolecular nature in principle favors the latter reaction.[35]

To exclude any adverse effects from additional compounds present in small amounts in the algae oil besides the fatty acids (*e.g.* carotenoids), ethenolysis of a mixture of model compounds (40% **FA16:0** 50%, **FA18:1** and 10% **FA20:5**) resembling the fatty acid composition of algae oil was investigated (Table 1, column "model substrate mixture"). The conversion and selectivity for all expected reaction products were determined over **FA16:0** as an internal standard *via* gas chromatography. The selectivity is defined as the ratio of the formed 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** (formation of two equivalents **CHD**).

In accordance to the experiments with neat methyl oleate, a catalyst loading of 0.5 mol% Hoveyda–Grubbs 1st generation catalyst (referring to the number of double bonds) and 10 bar of ethylene were chosen. After 6 h, a high conversion of 86% for **FA18:1** was observed (Table 1). This value is comparable to the conversion of neat methyl oleate as single model compound. The poly-unsaturated component **FA20:5** was almost completely consumed after 6 h.

The selectivity for the ethenolysis products of **FA18:1** (**HC10:1** and **E10:1**) was above 99%, as also observed for the single **FA18:1** compound. The ethenolysis product **E6:1** of **FA20:5** was also formed with a high selectivity of 85%. However, the self-metathesis of **FA20:5** could not be suppressed. The selectivity for the self-metathesis product 1,4-cyclohexadiene **CHD** was 84%. This suggests a higher reaction rate of the intramolecular self-metathesis leading to the formation of **CHD** compared to the intermolecular cross-metathesis.

Table 1 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 the self-metathesis product 1,4-cyclohexadiene (CHD)

Ethenolysis of		Model substrate mixture[a]	$scCO_2$ extracted algae oil
Composition of the initial reaction mixture [%]	**FA14:0**	—	9
	FA16:1	—	49
	FA16:0	40	27
	FA18:1	50	4
	FA18:0	—	2
	FA20:5	10	9
Conversion[b] [%]	**FA16:1**	—	81
	FA18:1	86	90
	FA20:5	>99	92
Selectivity[c] [%] for	**CHD**	84	64
	E6:1	85	82
	HC8:1	—	83
	HC10:1	>99	97
	E10:1	>99	84

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. Average of two independent experiments, *cf.* ESI for complete data. [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.

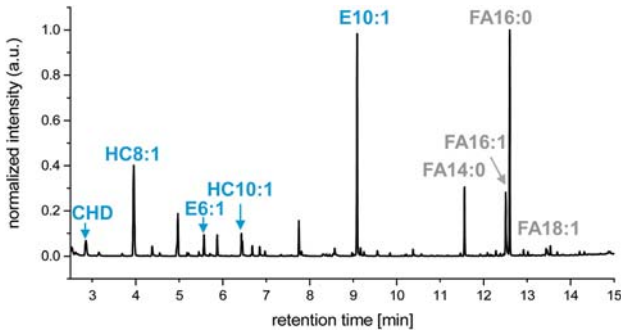


Fig. 4 Gas chromatogram of the ethenolysis of $scCO_2$ extracted algae oil in $scCO_2$ (after transesterification with methanol) with Hoveyda–Grubbs 1st generation catalyst and assignments of the ethenolysis products (HC8:1 1-octene, E6:1 methyl 5-hexenoate, HC10:1 1-decene, E10:1 methyl 9-decenoate), 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). Other signals likely originate from side-products of ethyl vinyl ether quenching.

Ethenolysis of algae oil was performed applying the same reaction conditions as for the model substrate mixture. The selectivities and conversions were determined *via* gas chromatography (Fig. 4) over **FA16:0** as internal standard, which is, besides **FA14:0**, found as saturated fatty acid in algae oil.

The conversion of the mono-unsaturated fatty acids in the algae oil are 81% and 90% for **FA16:1** and **FA18:1**, respectively, and are therefore in the same range as from the ethenolysis of the model substrate mixture. Also, the conversion of the fivefold unsaturated fatty acid **FA20:5** is again almost complete (92%). The selectivities for the ethenolysis products of the mono-unsaturated fatty acids are between 83% and 97% and consistently high compared to the model substrate mixture. The selectivity for **E6:1** is 82% and equals the result of the model substrate mixture. However, the selectivity of 64% for **CHD** as self-metathesis product is lower than the values observed with the model substrate mixture.

Overall, ethenolysis of $scCO_2$ extracted algae oil in $scCO_2$ proceeds with high conversions between 81% and 92% and high selectivities for ethenolysis products with exception of **CHD** formed by intramolecular self-metathesis of **FA20:5**.

Butenolysis of model compounds and microalgae oil

Besides ethenolysis, the cross-metathesis with internal alkenes such as 2-butene is a versatile tool to convert fatty acids to a wide spectrum of unsaturated compounds and gives access to products with chain lengths different to the ethenolysis products. While ethenolysis gives rise to products of mainly even-numbered hydrocarbon chains, butenolysis with 2-butene gives odd-numbered products. Furthermore, cross-metathesis with internal alkenes circumvents the specific disadvantage of ethenolysis: in the cross-metathesis with ethylene an unstable methylidene intermediate is formed which can lead to fast decomposition of the catalyst and therefore limited productivity.[36–38]

To establish the butenolysis in $scCO_2$ as reaction medium, first the butenolysis of methyl oleate as a model compound was investigated. Hoveyda–Grubbs 2nd generation catalyst was chosen as it gave better results in butenolysis reactions in organic solvent compared to Hoveyda–Grubbs 1st generation catalyst.[18,39,40] Similar to the aforementioned ethenolysis in $scCO_2$, the butenolysis was conducted at a pressure of 300 bar at a temperature of 45 °C.

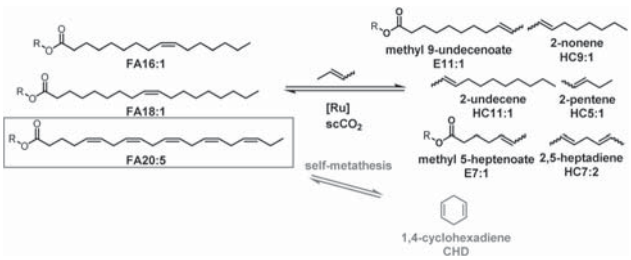
Butenolysis of methyl oleate results in the formation of methyl 9-undecenoate **E11:1** and 2-undecene **HC11:1** (*cf.* Scheme 1, R = Me). Conditions similar to butenolysis in dichloromethane were adopted (10 equivalents of 2-butene and 0.1 mol% Hoveyda–Grubbs 2nd generation catalyst),[18] which resulted in a conversion of methyl oleate of 93%. Only small amounts of self-metathesis products (dimethyl 9-octadecendioate **DE18:1** and 9-octadecene **HC18:1**) were formed. Selectivity for the desired butenolysis products was around 94%. The butenolysis products were formed in a *cis* : *trans* ratio of about 20 : 80. All these results match with butenolysis of methyl oleate in dichloromethane as solvent.[18,40] Upon decreasing the catalyst loading the selectivity was virtually unaffected whereas the conversion dropped to 82% at a catalyst loading of 0.05 mol% and to 25% at a catalyst loading of 0.01 mol%, respectively (Table S11†). Compared to ethenolysis of methyl oleate in $scCO_2$, the catalyst loading required for maximum butenolysis (0.1 mol%) is fivefold lower. As previously stated, the formation of the unstable methylidene intermediate cata-

lyst species in the ethenolysis is presumably a major factor contributing to the necessity of a higher catalyst loading. In addition, the more reactive terminal double bond of the ethenolysis products can compete with the internal one of the starting material.

Again, first the simplified model substrate mixture was used. The following butenolysis products were expected (Scheme 3): 2-undecene **HC11:1** and methyl 9-undecenoate **E11:1** from **FA18:1** and 2,5-heptadiene **HC7:2**, methyl 5-heptenoate **E7:1** and 2-pentene **HC5:1** formed from **FA20:5**. Note that complete butenolysis of **FA20:5** can generate up to four equivalents of **HC7:2**. 2-Pentene **HC5:1**, the smallest butenolysis product of **FA20:5**, was not detected in GC analysis due to its low boiling point.

The same reaction conditions as for methyl oleate were adopted employing a catalyst loading of 0.1 mol% Hoveyda–Grubbs 2nd generation catalyst (referring to the number of double bonds). After two hours, GC analysis revealed almost complete conversion for **FA20:5** and a conversion of 88% for **FA18:1**. Butenolysis of **FA18:1** proceeded in high selectivity (88% for **E11:1** and 84% for **HC11:1**) whereas **FA20:5** was converted somewhat less selective (**HC7:2** 52% and **E7:1** 66%). Incomplete butenolysis of **FA20:5**, leading to two-, three- and four-fold unsaturated products, can decrease the selectivity for **E7:1** and **HC7:2**. Remarkably, the selectivity for the self-metathesis product **CHD** is only 27% and thus significantly lower compared to the corresponding ethenolysis experiment. Please note that the concentrations of 2-butene and ethylene differ. In the butenolysis a 10-fold excess of 2-butene was applied, whereas in the ethenolysis experiments a 4-fold excess of ethylene was used.

Based on these results, butenolysis of $scCO_2$ extracted algae oil (Table 2, column "$scCO_2$ extracted algae oil" and Fig. 5) was carried out applying the same reaction conditions (0.1 mol% catalyst, 300 bar $scCO_2$ at 45 °C, 10-fold excess of 2-butene). Besides 2-nonene **HC9:1**, 2-undecene **HC11:1** and methyl 9-undecenoate **E11:1** as butenolysis products from **FA16:1** and **FA18:1**, respectively, methyl 5-heptenoate **E7:1** and 2,5-heptadiene **HC7:2** from **FA20:5** were found. With 91% and 99% the conversions of **FA18:1** and the five-fold unsaturated fatty **FA20:5**, respectively, agree with the conversions observed for the mixture of model compounds (Table 2). For **FA16:1** a conversion of 81% was determined. The selectivities for the butenolysis products of the mono-unsaturated fatty acids are high (between 82 and 85%) and in the same range as for methyl oleate as a single model component. Furthermore, the butenolysis products of **FA20:5** are formed with selectivities comparable to the simplified model substrate mixture.



Scheme 3 Butenolysis products of the unsaturated fatty acids present in algae oil from *Phaeodactylum tricornutum*, and CHD as a self-metathesis product of FA20:5.

Table 2 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 the self-metathesis product 1,4-cyclohexadiene (CHD)

Butenolysis of		Model substrate mixture[a]	$scCO_2$ extracted algae oil
Composition of the initial reaction mixture [%]	**FA14:0**	—	9
	FA16:1	—	47
	FA16:0	40	25
	FA18:1	50	8
	FA18:0	—	1
	FA20:5	10	10
Conversion[b] [%]	**FA16:1**	—	81
	FA18:1	88	91
	FA20:5	97	>99
Selectivity[c] [%] for	**CHD**	27	24
	HC7:2	52	55
	E7:1	66	65
	HC9:1	—	85
	HC11:1	84	83
	E11:1	88	82

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. Average of two independent experiments, *cf.* ESI for complete data. [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.

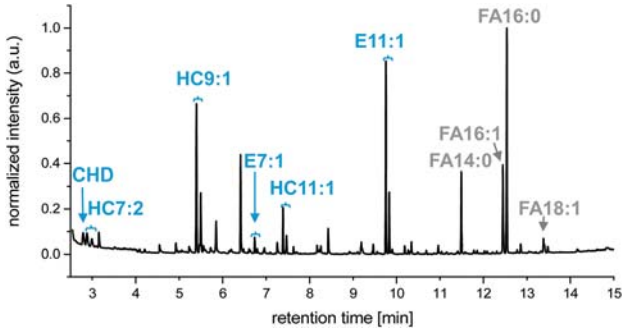


Fig. 5 Gas chromatogram of butenolysis in $scCO_2$ of $scCO_2$ extracted algae oil (after transesterification with methanol) with Hoveyda–Grubbs 2nd generation catalyst and assignments of the butenolysis products (HC7:2 2,5-heptadiene, HC9:1 2-nonene, E7:1 methyl 5-heptenoate, HC11:1 2-undecene, E11:1 methyl 9-undecenoate) and the fatty acid esters (FA14:0 methyl myristate, FA16:1 methyl palmitoleate, FA16:0 methyl palmitate, FA18:1 methyl oleate). Other signals likely originate from side-products of ethyl vinyl ether quenching.

All in all, in the butenolysis of $scCO_2$ extracted algae oil high conversions and selectivities were achieved comparable to those obtained in the reactions with the neat model substances.

Simultaneous extraction and cross-metathesis in $scCO_2$

As demonstrated above, $scCO_2$ is a powerful extraction medium for lipids of microalgae and is also a suitable reaction medium for cross-metathesis of this algae feedstock that is compatible with the ruthenium-based catalysts. In terms of reducing multiple reaction steps, avoiding solvent removal and integrating a direct valorization of the feedstock, we propose a combined approach of extraction and cross-metathesis of microalgae in $scCO_2$.

The combination of extraction and ethenolysis or butenolysis was performed at a $scCO_2$ pressure of 300 bar at 45 °C. For both catalytic transformations, 1 g of ultrasound pre-treated freeze-dried algae were placed in a high-pressure reactor together with the corresponding amount of the metathesis catalyst (0.5 mol% of Hoveyda–Grubbs 1st generation catalyst for ethenolysis and 0.1 mol% Hoveyda–Grubbs 2nd generation catalyst for butenolysis, respectively). In case of ethenolysis, 10 bar ethylene and for butenolysis, a 10-fold excess of 2-butene was applied.

Subjecting the freeze-dried algae to the combined extraction and ethenolysis results in a conversion of 32% and 44% (Table 3) for **FA16:1** and **FA18:1**, respectively. In contrast, the conversion of the fivefold unsaturated fatty acid **FA20:5** is higher with 88%.

In comparison to the two-step procedure the conversions in this combined approach differ significantly. While the conversion of the poly-unsaturated fatty acid **FA20:5** is almost identical (88% for combined and 92% for two-step approach), the mono-unsaturated fatty acids **FA16:1** and **FA18:1** are converted to a much lower extent. This might be due to a slow extraction over the duration of the experiment, such that the extracted fatty acids are not exposed to the catalyst over the entire experiment. One reason for this are different high-pressure setups. In the combined approach the algae oil is extracted by means of a static $scCO_2$ batch reactor, whereas in the two-step process the algae oil is extracted under a continuous flow. Furthermore, reaction conditions had to be adopted to the limited thermal stability of the Ru catalyst and were not ideal for a maximum yield of extracted oil.

However, the selectivities in the combined approach are comparable to the two-step procedure. The selectivities for the ethenolysis products of the mono-unsaturated fatty acids in the combined approach are between 60 and 88% and in the two-step procedure between 83 and 97%. Also, for the poly-unsaturated fatty acid **FA20:5** the selectivities for the ethenolysis product **E6:1** (87%) and for the self-metathesis product **CHD** (49%) are comparable to the extraction and separate metathesis. All in all, the characteristic selectivity of the catalyst is evidently not affected by components of the freeze-dried algae.

In the combined extraction and butenolysis of pre-treated freeze-dried algae (Table 3) again lower conversions of the mono-unsaturated fatty acids were observed. Conversions of 47% and 65% were found for **FA16:1** and **FA18:1**, respectively. The conversion of the poly-unsaturated fatty acid **FA20:5** of 89% is still in the same range as for the butenolysis two-step process.

The desired butenolysis products of the mono-unsaturated fatty acids **FA16:1** and **FA18:1** are formed with selectivities between 70 and 97%. For **E7:1** a selectivity of 67% is observed. These are again in accordance with the two-step approach.

The conformity of trends in conversions in ethenolysis and butenolysis, respectively, in the combined approaches shows that the combination of extraction and catalytic transformation in one batch reactor is feasible but requires optimization in terms of extraction. However, it is important to note that this integrated one-pot approach shows comparable selectivities for the desired higher-value chemicals in relation to the step-wise approach of extraction and catalysis, and therefore, makes this a promising concept to overcome the typical bottleneck of extraction in the valorization of biomass.

Table 3 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	
Conversion[c] [%]	**FA16:1**	32	**FA16:1**	47
	FA18:1	44	**FA18:1**	65
	FA20:5	88	**FA20:5**	89
Selectivity[d] [%] for	**CHD**	49	**CHD**	36
	HC5:2[e]	—	**HC7:2**	70
	E6:1	87	**E7:1**	67
	HC8:1	60	**HC9:1**	96
	HC10:1	88	**HC11:1**	97
	E10:1	77	**E11:1**	70

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. Average of two independent experiments, *cf.* ESI for complete data. [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.

Alkoxycarbonylation

To further illustrate the concept of integrated $scCO_2$ extraction and functionalization of fatty acids from microalgae, isomerizing alkoxycarbonylation was studied as another catalytic transformation system that enables the upgrading of algae oil into higher value chemical intermediates. This converts an internal double bond into a terminal ester group by reaction with methanol and CO. Pd(II) catalysts with a sterically demanding

diphosphine ligand such as [Pd(dtbpx)(OTf)$_2$] (dtbpx = 1,2-bis ((di-*tert*-butylphosphino) methyl)benzene) convert unsaturated fatty acid esters such as methyl oleate to linear long-chain α,ω-diesters with high linear selectivities (>90%).[41,42] As demonstrated previously, mid-chain (di-)carboxylic acid esters, currently only accessible *via* demanding synthetic routes, can be obtained in a two-step fully catalytic route by butenolysis and subsequent isomerizing alkoxycarbonylation without prior work-up and purification.[18] Thus, in order to prove the feasibility of a bio-refinery concept starting from crude microalgae biomass, scCO_2 extraction was combined with isomerizing alkoxycarbonylation.

Using methyl oleate as model compound, the isomerizing alkoxycarbonylation was performed at 90 °C and 30 bar CO pressurized with CO_2 to a total pressure of 325 bar with additional methanol. As a catalyst system 0.8 mol% [Pd(dtbpx)(OTf)$_2$] and 4 mol% (dtbpxH_2)(OTf)$_2$ was used. After 18 h, a conversion of 29% was observed which is lower than expected under standard conditions applying pure methanol as solvent.[41,43] This is due to the low nucleophile concentration which is rate determining.[44] Yet the high selectivity for the linear product (92%) was not affected.

The isomerizing alkoxycarbonylation of scCO_2 extracted algae oil under identical conditions (but with a reaction time of 96 h, affording a conversion of 50%) again, proceed with high selectivities around 90% for both, the linear 1,19-diester from **FA18:1** and the linear 1,17-diester from **FA16:1**.

Also, in the one-pot combination of extraction and isomerizing alkoxycarbonylation of the lipids from freeze-dried algae, the fatty acids were converted to the desired linear diester with selectivities over 90%. Overall, these findings underline the viability of this reaction in supercritical CO_2.

Conclusions

An integration of biomass extraction and catalytic upgrading in supercritical CO_2 as a solvent allows for a more straightforward utilization of microalgae biomass as a source of chemical intermediates. The desired lipids are extracted selectively and converted in a one-pot approach by catalytic olefin ethenolysis or butenolysis, respectively, to target mid-chain olefins and unsaturated esters. These olefin metathesis in scCO_2 proceed with high conversions and selectivities. In addition to its advantageous selectivity as a solvent, CO_2 is benign and easy to remove from the products.

The approach of integrated extraction and catalytic upgrading of lipids, demonstrated here for the case of olefin metathesis and isomerizing carbonylation, can help to overcome the bottleneck biomass extraction represents for its utilization as a feedstock, and of microalgae in particular.

Conflicts of interest

There are no conflicts to declare.

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