Olefin Metathesis of Microalgae Lipids

Dissertation

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Publications

Parts of this thesis have been published

Zimmerer, J.; Williams, L.; Pingen, D.; Mecking, S., Green Chem. **2017**, 19, 4865-4870. "Mid-chain carboxylic acids by catalytic refining of microalgae oil."

Zimmerer, J.; Pingen, D.; Hess, S. K.; Koengeter, T.; Mecking, S., Green Chem. **2019**, 21, 2428-2435. "Integrated extraction and catalytic upgrading of microalgae lipids in supercritical carbon dioxide."

Publication related to this work

Pingen, D.; Zimmerer, J.; Klinkenberg, N.; Mecking, S., Green Chem. **2018**, 20, 1874-1878. "Microalgae lipids as a feedstock for the production of benzene."

Zusammenfassung

Mikroalgen sind eine attraktive alternative Lipidquelle zu den typischen Ölpflanzen wie Raps, Sonnenblumen oder Sojabohnen, da sie eine sehr hohe Wachstumsrate aufweisen und dadurch deutlich effizienter Öl produzieren. Ihr Ölanteil kann bei bis zu 70% liegen. Für ihre Kultivierung wird weder landwirtschaftliche Nutzfläche noch Trinkwasser benötigt. Mikroalgen können sowohl in Salz- wie auch Brackwasser kultiviert werden, somit entfällt die Konkurrenz zur Lebensmittelproduktion. Neben den Vorteilen in der Kultivierung und Effektivität, produzieren Algen zudem besondere, in typischen Ölpflanzen weniger vorkommende, Fettsäuren wie beispielsweise Palmitoleinsäure oder auch mehrfach ungesättigte Fettsäuren wie Eicosapentaensäure und Docosahexaensäure. Speziell diese ω-3-Fettsäuren haben durch ihre Verwendung als Nahrungsergänzungsmittel an Bedeutung gewonnen.

In dieser Arbeit wurde die einzellige Kieselalge *Phaeodactylum tricornutum* als Lipidquelle verwendet, deren enthaltene Lipide mittels organischer Lösungsmittel gemäß einer Methode nach Folch extrahiert wurden. Dabei wurden aus einer 30 L Algenkultur 6-7 g Algenöl erhalten, welches nach einer Umesterung mit Methanol mittels Gaschromatographie analysiert und die Fettsäurenzusammensetzung bestimmt wurde (**Tabelle 1, Abbildung 1**).

Tabelle 1: Fettsäurezusammensetzung des vom Mikroalgenstamm *Phaeodactylum tricornutum* extrahierten Öls.

Fettsäuremethylester	**Gehalt [%][a]**
Methylmyristat (FA14:0)	8
Methylpalmitoleat (FA16:1)	40
Methylpalmitat (FA16:0)	32
Methyloleat (FA18:1)	10
Methyleicosapentaenoat (FA20:5)	9

[a]Der Gehalt wurde gaschromatographisch nach Umesterung mit Methanol bestimmt.

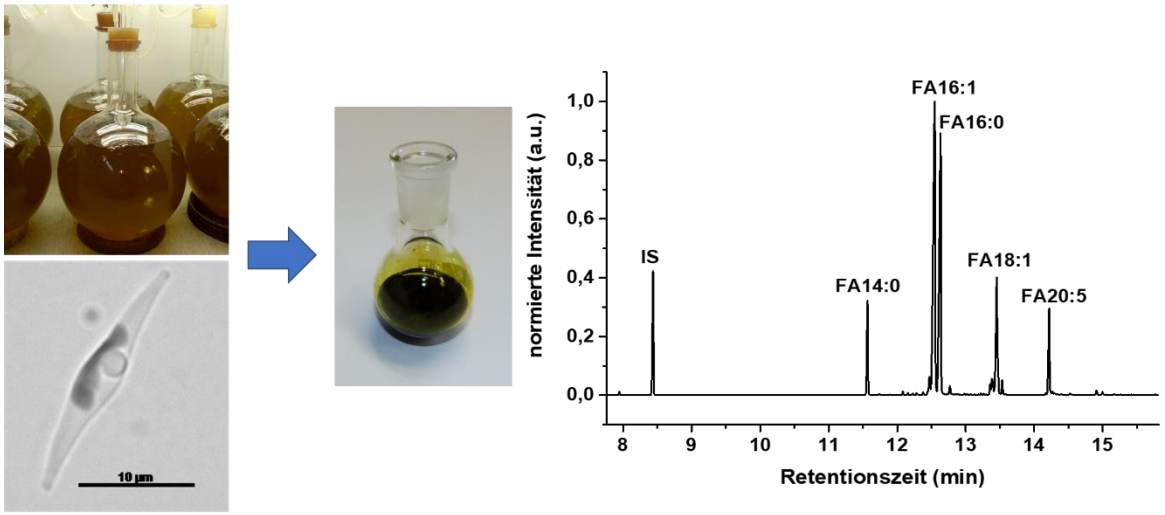

Abbildung 1: Gaschromatogramm des von *Phaeodactylum tricornutum* extrahierten Algenöls mit Nonansäure als internen Standard (IS) (nach der Veresterung mit Methanol). Die Integrale der Signale sind in Tabelle 1 aufgeführt.

Derzeit liegt der Fokus der Forschung auf der Verwendung von Algenöl als Ausgangsmaterial für die Biodieselproduktion, was jedoch das Potential dieses besonderen Rohstoffs und die einzigartige Struktur der Fettsäuren nicht ausschöpft. Aufgrund der langen Kohlenwasserstoffkette, der Carboxylgruppe und der enthaltenen Doppelbindung(en) sind ungesättigte Fettsäuren für katalytische Funktionalisierungen besonders interessant. In dieser Arbeit dient das Algenöl als Ausgangsmaterial für eine Bioraffinerie basierend auf Metathese und anschließender isomerisierenden Alkoxycarbonylierung zur Herstellung von (Di-)Estern mit mittlerer Kettenlänge, welche aktuell nur über aufwändige Synthesewege zugänglich sind. Zunächst wurden kommerziell erhältliche Fettsäureester als Modellsubstanzen in einer Kreuz-Metathese Reaktion mit 2-Buten als Reaktionspartner (Butenolyse) katalytisch umgesetzt, um dabei geeignete Reaktionsbedingungen zu finden und um mögliche Produkte zu identifizieren. In der Butenolyse der einfach- und mehrfach ungesättigten Fettsäureester, im einzelnen Methylpalmitoleat (FA16:1), Methyloleat (FA18:1) und Methyleicosapentaenoat (FA20:5), wurden verschiedene ungesättigte Ester sowie Mono- und Diene in hohen Selektivitäten erhalten. Bemerkenswert ist dabei die selektive Umsetzung des fünffach ungesättigten Fettsäureesters FA20:5 zu vier Äquivalenten 1,5-Heptadien. Die erhaltenen Produktmischungen wurden durch eine isomerisierende Alkoxycarbonylierung weiter zu den entsprechenden linearen (Di-) Carbonsäureester mit mittlerer Kettenlänge umgesetzt. Basierend auf den Ergebnissen der verschiedenen Modellsubstanzen wurde auch Algenöl in diesem zweitstufigen Reaktionsprozess umgesetzt. Bemerkenswerterweise erwies sich der dabei verwendete Metathesekatalysator (Hoveyda-Grubbs Katalysator der zweiten Generation) als kompatibel mit den verschiedenen im Algenöl enthaltenen Komponenten wie z. B. Diacylglyceride oder Chlorophyll. Die enthaltenen

ungesättigten Fettsäuren wurden in dieser Reaktion nahezu vollständig umgesetzt (98% für FA16:1 und FA18.1 sowie 100% für FA20:5). Anschließend wurde die Butenolysereaktionsmischung ohne weitere Aufarbeitung in einer isomerisierenden Alkoxycarbonylierung innerhalb von 3 Tagen zu den entsprechenden (Di)Estern umgesetzt (**Abbildung 2**). Auch hierbei erwies sich der eingesetzte Katalysator [Pd(dtbpx)(OTf)$_2$] als kompatibel mit der vorliegenden Vielkomponentenmischung sowie dem zuvor gezielt deaktivierten Metathesekatalysator. Für beide Reaktionsschritte wurden hohe Selektivitäten für die erwünschten Produkte beobachtet.

Mit diesem Konzept werden verschiedene Mono- und Diester mit Kettenlängen zwischen C8 und C12 basierend auf Mikroalgenöl als erneuerbarem Rohstoff zugänglich. Die resultierenden Carbonsäureester können als Tenside (E12:0 und E10:0) oder Nahrungsmittelzusätze (E6:0) verwendet werden. Zudem können Dicarbonsäureester als Schmiermittel oder als Monomere in Polykondensationsreaktionen eingesetzt werden. Als weiteres Produkt dieses Konzeptes ist der Azelainsäureester besonders interessant, da dessen derzeitige industrielle Produktion auf der technisch anspruchsvollen und potenziell gefährlichen Ozonolyse basiert. Bei dem hier gezeigten zweitstufigen katalytischen Prozess wird das gesamte Fettsäurespektrum des Algenöls genutzt.

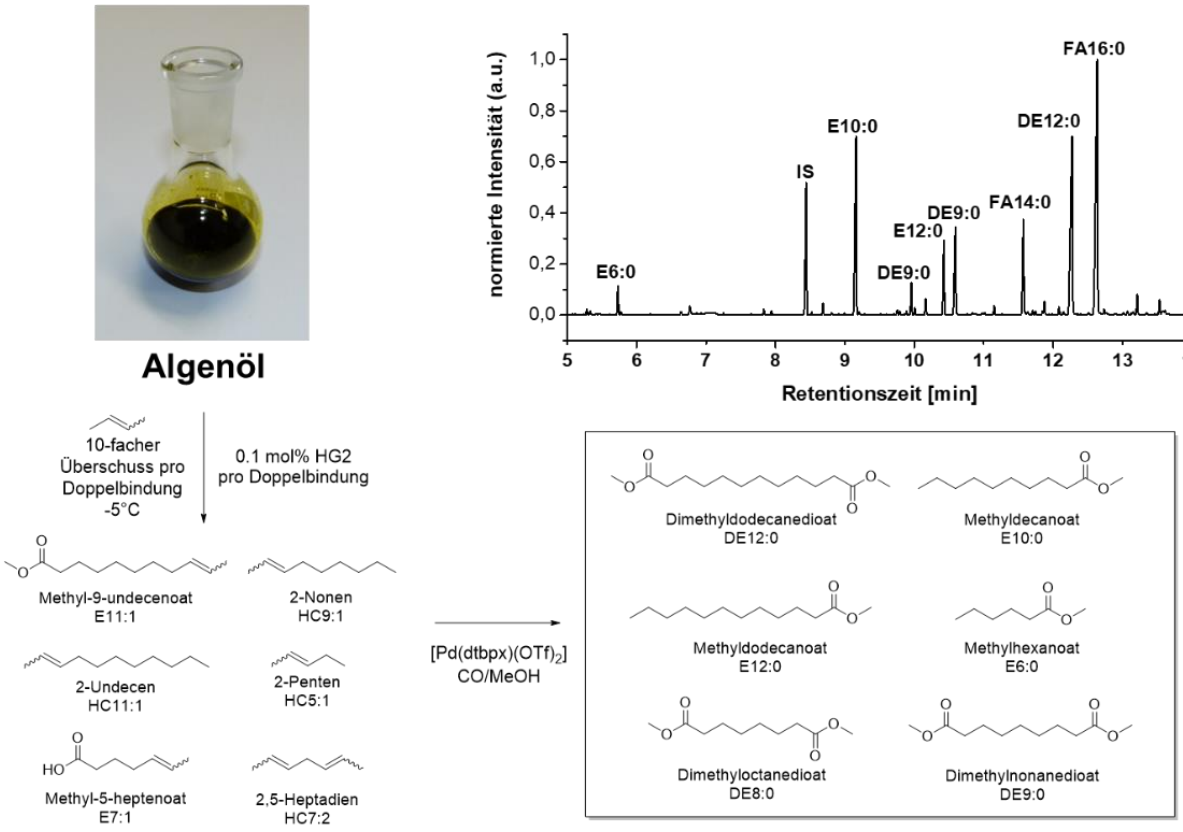

Abbildung 2: Gaschromatogramm der methoxycarbonylierten Butenolyseprodukte von Algenöl und die entsprechend zugeordneten Produkte.

Gegenüber der Butenolyse bietet die Kreuzmetathese mit Ethylen (Ethenolyse) eine gute Möglichkeit, um ein breites Spektrum an Produkte mit terminalen Doppelbindungen zu erhalten. Auch hier wurden zunächst kommerziell erhältliche Fettsäure(-ester) als Modelsubstanzen eingesetzt und untersucht, bevor organisch extrahiertes Algenöl mit Hoveyda-Grubbs Katalysator der ersten Generation bei 1,5 bar Ethylen Druck umgesetzt wurde (**Abbildung 3**). Dabei erwies sich der verwendete Katalysator als kompatibel mit den verschiedenen Komponenten des Algenöls. Für die im Algenöl enthaltenen einfach ungesättigten Fettsäureester (FA16:1 und FA18:1) wurden Umsätze von 67% beziehungsweise 69% erzielt, wobei FA20:5 sogar zu 89% umgesetzt wurde. Die gewünschten terminalen Produkte wurden mit einer hohen Selektivität gebildet, nur die intramolekulare Selbst-Metathese von FA20:5, durch die 1,4-Cyclohexadien gebildet wird, konnte nicht unterdrückt werden.

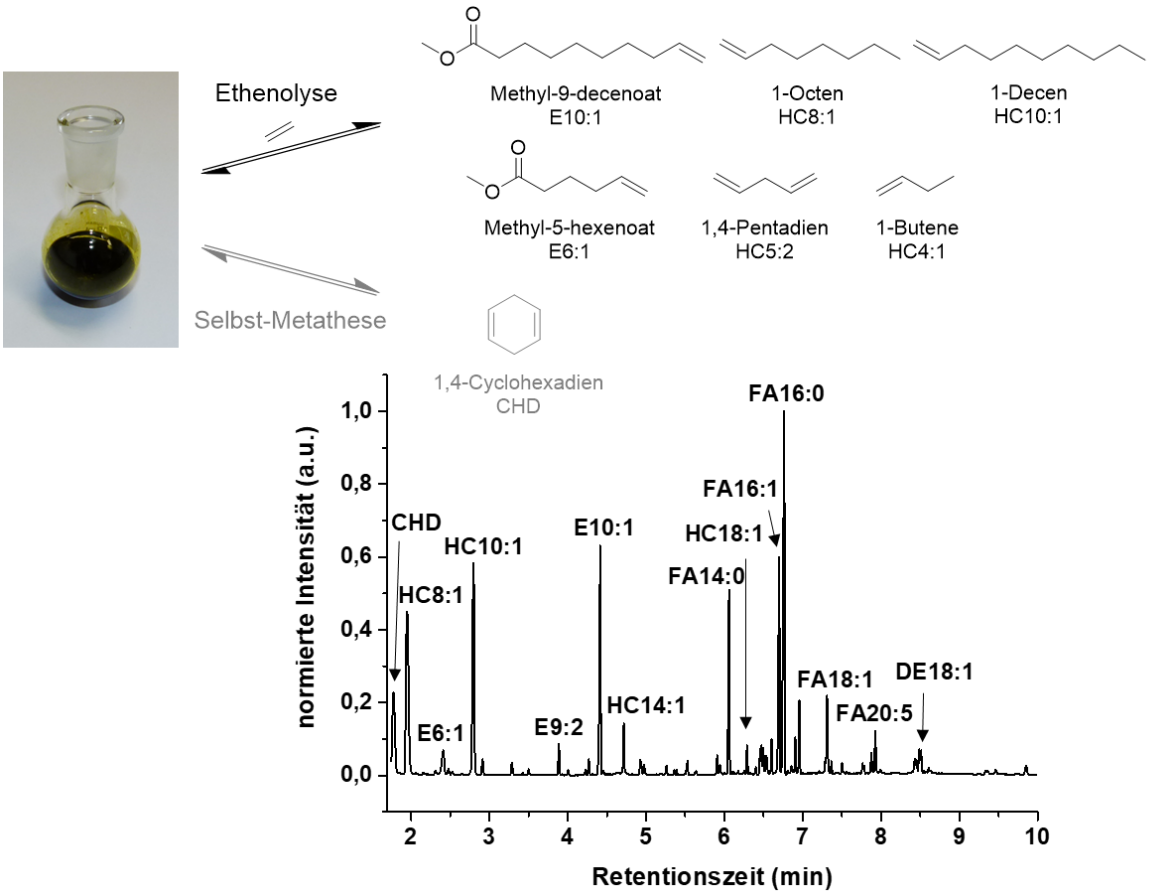

Abbildung 3: Gaschromatogramm der Reaktionsmischung der Ethenolyse von Algenöl (nach der Veresterung mit Methanol).

In der Natur kommen nur bestimmte Fettsäuren vor, weswegen das Produktspektrum, welches durch einer Ethenolyse erhalten werden kann, begrenzt ist. Durch die Kombination von Isomerisierung der Doppelbindung und Ethenolyse kann die Produktvielfalt gesteigert werden. Im Rahmen dieser Arbeit wurde die isomerisierende Ethenolyse von Methyloleat mit gängigen

N-heterocyclischen Carben (NHC) basierten Ru Metathesekatalysatoren untersucht, wobei als zusätzlicher Isomerisierungskatalysator [Pd(dtbpx)(OTf)$_2$] eingesetzt wurde. Dabei liegt der Fokus auf Produkten mit Kettenlängen kürzer als 10 Kohlenstoffatome, welche durch eine erste, Ketten verkürzende Ethenolysereaktion und anschließende isomerisierende Ethenolyse erst möglich werden (**Abbildung 4**, blauer Kasten). Für diesen zweistufigen Prozess wurden sowohl HG1 als auch HG2 als Metathesekatalysatoren untersucht, wobei deren Aktivität negativ vom zusätzlich verwendeten [Pd(dtbpx)(OTf)$_2$] beeinflusst wurde. Im Fall von HG2 konnten jedoch durch eine nachträgliche Zugabe von Metathesekatalysator die isomerisierten Produkte weiter in einer Metathese umgesetzt werden, wobei eine komplexe Produktmischung erhalten wurde.

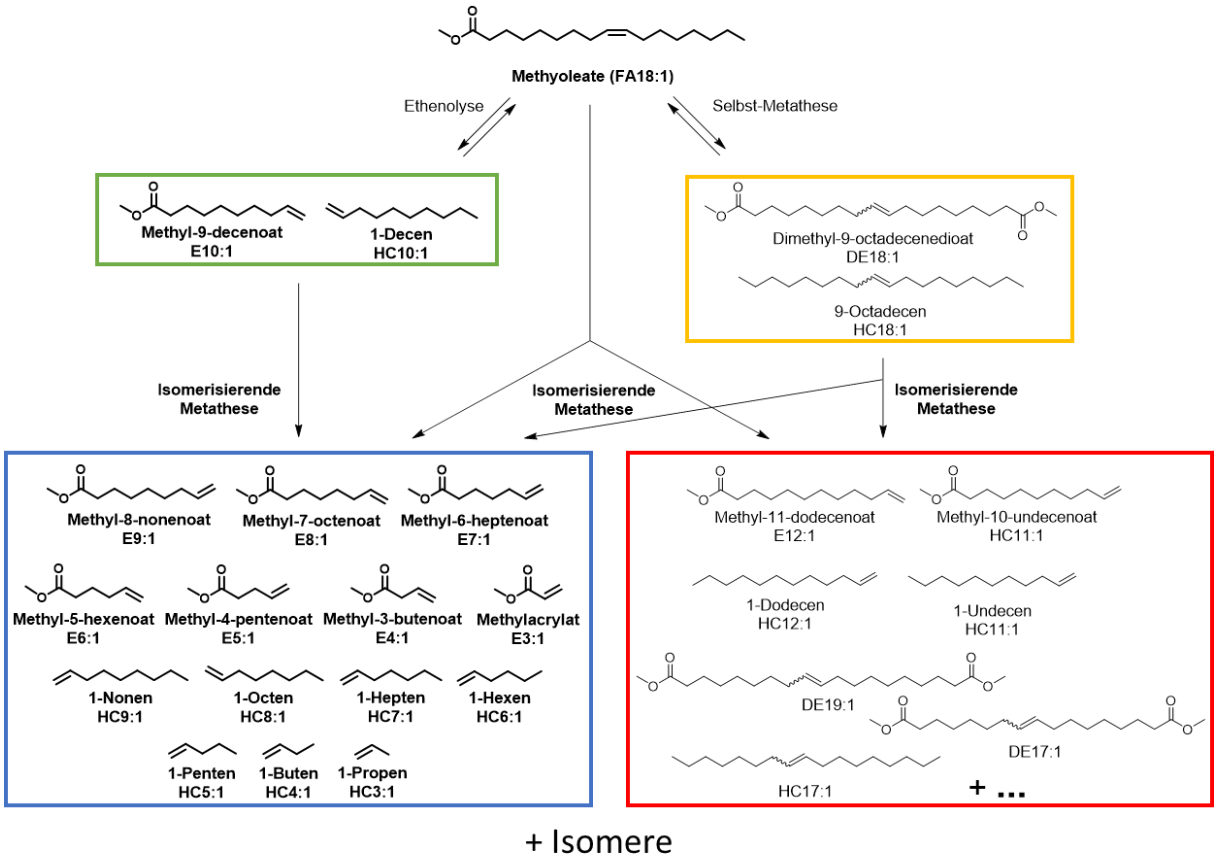

Abbildung 4: Schematische Produktübersicht der isomerisierenden Ethenolyse: Primäre Ethenolyseprodukte (grüner Kasten), primäre Selbst-Metatheseprodukte (gelber Kasten), Produkte der Isomerisierung und Metathese mit C<10 (blauer Kasten), Produkte der Isomerisierung und Metathese mit C>10 (roter Kasten).

Bis zu diesem Punkt wurde gezeigt, dass die aus Mikroalgen gewonnenen Fettsäuren katalytisch zu höherwertigen Chemikalien umgesetzt werden können. Jedoch erfolgt deren Extraktion in mehreren energieaufwändigen Schritten, wobei organische Lösungsmittel zum Einsatz kommen. Dies kann durch eine Kombination von Extraktion und katalytischer Funktionalisierung (Ethenolyse oder Butenolyse) umgangen werden, wofür überkritisches

Kohlenstoffdioxid ($scCO_2$) ein geeignetes Lösungsmittel darstellt. Für dieses Eintopfverfahren wurde gefriergetrocknete Biomasse der Mikroalge *Phaeodactylum tricornutum* verwendet.

Zunächst wurde die Lipidextraktion in $scCO_2$ untersucht, dazu wurde sowohl der Druck als auch die Temperatur und damit die Dichte variiert. Im Allgemeinen konnten die erzielten Ausbeuten durch Vorbehandlung der Biomasse mittels Ultraschall gesteigert werden. Unter optimierten Bedingungen (90 °C, 620 bar, $\rho(CO_2)$ = 0.90 g mL^{-1}, **Abbildung 5**) wurden mit einer Ausbeute von 25 Gew.-% selektiv und quantitativ die in der Biomasse vorhandenen Lipide extrahiert. Auch die Extraktion mit organischen Lösungsmitteln verlief quantitativ (28 Gew.-%), war jedoch weniger selektiv. Es wurden zusätzlich polare Diacylglyceride und Chlorophyll abgetrennt.

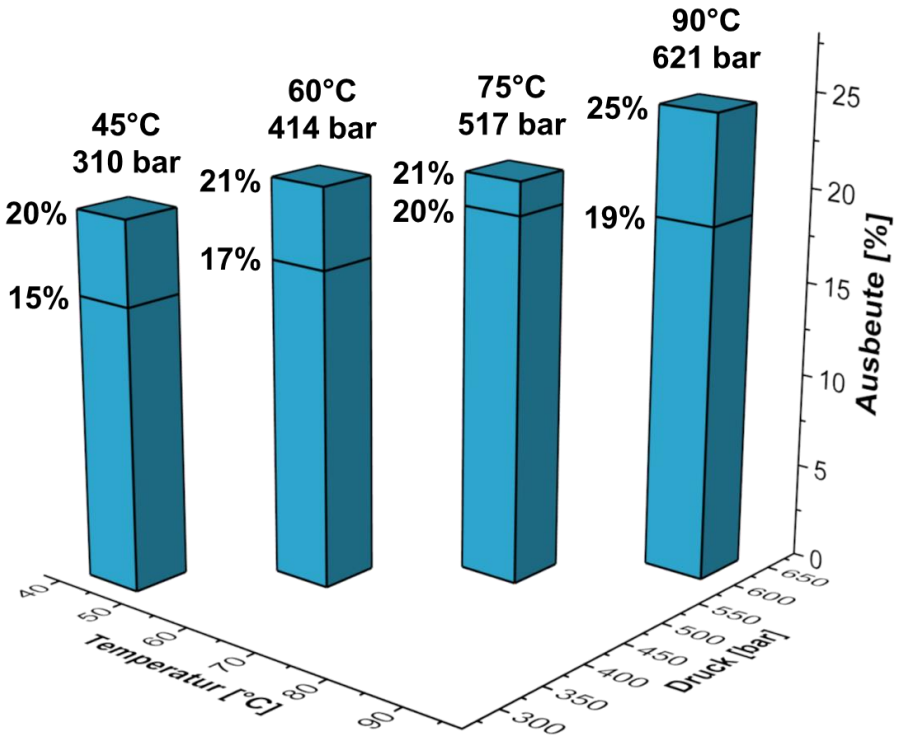

Abbildung 5: Ausbeuten der $scCO_2$ Extraktion bei verschiedenen Drücken und Temperaturen bei konstanter Dichte (0,9 g mL^{-1}). Unterer Teil der Balken: Extraktion von gefriergetrockneten Algen, gesamter Balken: Extraktion von Ultraschall vorbehandelten, gefriergetrockneten Algen.

Um geeignete Bedingungen für die kombinierte Extraktion und Funktionalisierung zu finden, wurden zunächst Methyloleat als Modellverbindung sowie eine Fettsäureestermischung angelehnt an die Zusammensetzung des Algenöls untersucht. Bei 45 °C und einem Gesamtdruck von 300 bar stellte sich für die Ethenolysis der Hoveyda-Grubbs Katalysator der ersten Generation sowie für die Butenolyse der Hoveyda-Grubbs Katalysator der zweiten Generation als geeignet heraus. Auf diesen Ergebnissen aufbauend konnte sowohl die Ethenolyse als auch die Butenolyse in $scCO_2$ mit getrennt extrahiertem Algenöl erfolgreich durchgeführt werden. Die ermittelten

hohen Umsätze und Selektivitäten sind vergleichbar mit denen der Modellverbindungen. Um die Anzahl der nötigen Reaktionsschritte zu verringern und um das Entfernen von Lösungsmittel zu vermeiden, wurde die Extraktion der Fettsäure(-ester) und deren Umsetzung in einer Metathese-reaktion kombiniert. Dadurch wurden die gewünschten Alkene und ungesättigten Ester direkt aus der Biomasse „Mikroalge“ zugänglich (**Abbildung 6**). Auch hierbei entsprach die Produkt-zusammensetzung derer der Modellverbindungen wie auch derer des getrennt extrahierten Algenöls.

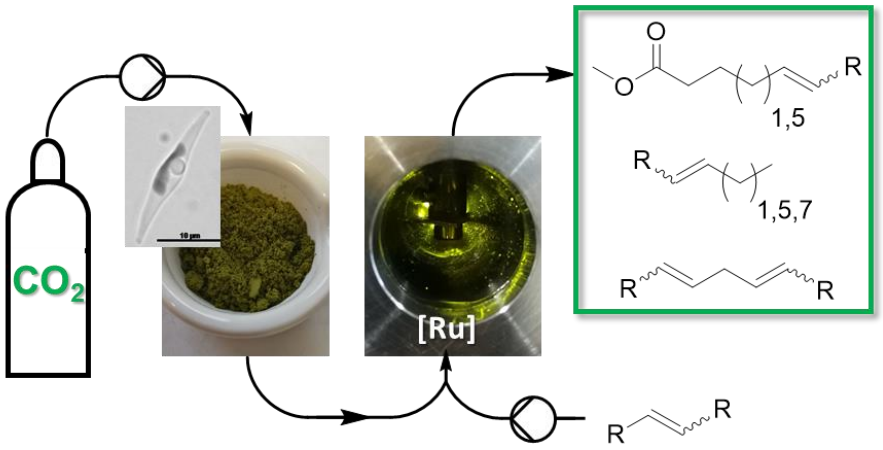

Abbildung 6: Schematische Darstellung der kombinierten Extraktion und Funktionalisierung via Kreuz-Metathese in $scCO_2$.

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Annotations

Labelling of Compounds

G1	Grubbs 1st generation catalyst
G2	Grubbs 2nd generation catalyst
G3	Grubbs 3rd generation catalyst
HG1	Hoveyda-Grubbs 1st generation catalyst
HG2	Hoveyda-Grubbs 2nd generation catalyst
UM73 SIPr	Umicore M73 SIPr catalyst
UM2	Umicore M2 catalyst
HC7:2	2,5-Heptadiene
HC8:1	1-Octene
HC8:2	2,5-Octadiene
HC9:1	2-Nonene
HC10:0	Decane
HC10:1	1-Decene
HC10:3	2,5,8-Decatriene
HC11:0	Undecane
HC11:1	2-Undecene
HC11:3	2,5,8-Undecatriene
HC13:0	Tridecane
HC13:4	2,5,8,11-Tridecatetraene
HC14:0	Tetradecane
HC14:1	7-Tetradecene
HC14:4	2,5,8,11-Tetradecatetraene
HC16:0	Hexadecane
HC16:5	2,5,8,11,14-Hexadecapentaene
HC17:0	Heptadecane
HC17:5	2,5,8,11,14-Heptadecapentaene
HC18:1	9-Octadecene

E5:1	Methyl 4-pentenoate
E6:0	Methyl hexanoate
E7:0	Methyl Heptanoate
E7:1	Methyl 5-heptenoate
E8:0	Methyl octanoate
E8:1	Methyl 5-octenoate
E9:0	Methyl nonanoate
E9:2	Methyl 5,8-nonadienoate
E10:0	Methyl decanoate
E10:1	Methyl 9-decenoate
E10:2	Methyl 5,8-decadienoate
E11:0	Methyl undecanoate
E11:1	Methyl 9-undecenoate
E11:2	Methyl 5,8-undecadienoate
E13:0	Methyl tridecanoate
E12:0	Methyl dodecanoate
E12:3	Methyl 5,8,11-dodecatrienoate
E14:0	Methyl tetradecanoate
E15:0	Methyl pentadecanoate
E16:4	Methyl 5,8,11,14-hexadecatetraenoate
E19:0	Methyl nonadecanoate
E19:5	Methyl 5,8,11,14,17-nonadecapentaenoate
E20:0	Methyl eicosanoate
DE8:0	Dimethyl octanedioate
DE9:0	Dimethyl nonanedioate
DE10:0	Dimethyl decandioate
DE10:1	Dimethyl 5-decendioate
DE12:0	Dimethyl dodecanedioate
DE18:1	Dimethyl 9-octadecenedioate

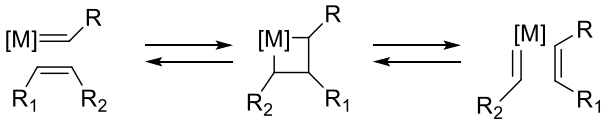
Abbreviations

P. tricornutum	*Phaeodactylum tricornutum*
$scCO_2$	Supercritical carbon dioxide
RCM	Ring-closing metathesis
ROMP	Ring-opening metathesis
ADMET	Acyclic diene metathesis
SHOP	Shell Higher Olefin Process
IS	Internal standard
GC	Gas chromatography
CAAC	Cyclic alkyl amino carbene
TON	Turnover number

1 General Introduction

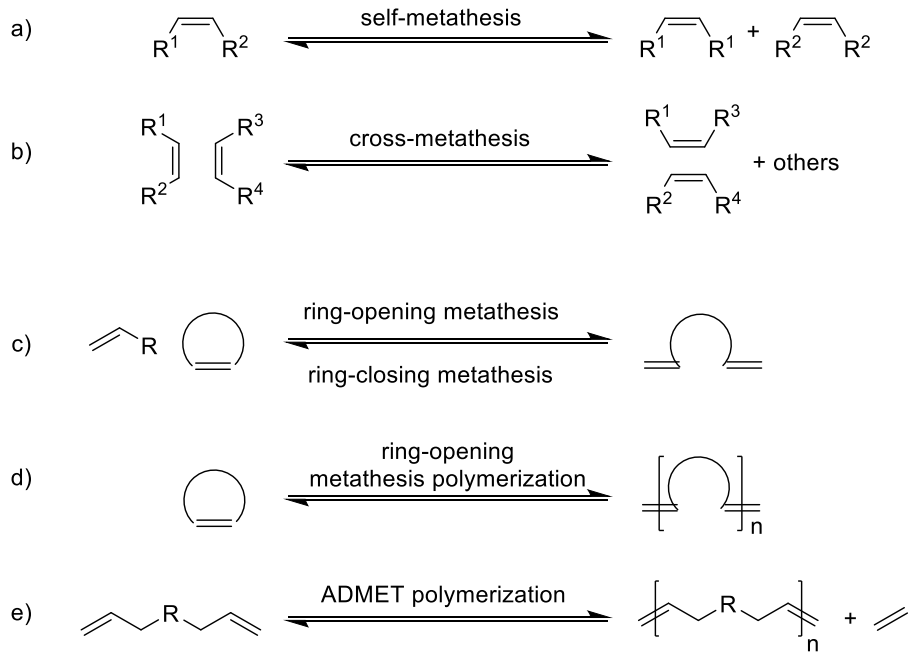
1.1 Olefin Metathesis

Olefin metathesis plays a central role in the organic synthesis and industry since its discovery in the 1960s.[1] In a typical reaction, a carbon-carbon double bond is cleaved and reformed. In this way substituent groups are exchanged, and an equilibrium distribution of metathesis products is obtained. Hérisson and Chauvin were the first to suggest the now generally accepted mechanism proposing a metal alkylidene complex as catalytically active species and a metallacyclobutane intermediate of the olefin metathesis equilibrium (**Scheme 1-1**).[2, 3]



Scheme 1-1: Mechanism of olefin metathesis proposed by Hérisson and Chauvin.

Various types of olefin metathesis are possible (**Scheme 1-2**) and generally, self-metathesis and cross-metathesis are distinguished. In a self-metathesis reaction (**Scheme 1-2**, a), two identical olefins react with each other, whereas in a cross-metathesis reaction (**Scheme 1-2**, b), two different olefins are involved. Self-metathesis is also possible in an intramolecular manner if the olefin contains more than one double bond. An important example and application of an intramolecular self-metathesis is ring-closing metathesis (RCM, **Scheme 1-2**, c) of dienes. The back-reaction, the so-called ring-opening metathesis (ROM), is its cross-metathesis counterpart. A special case of ROM is the polymerization of cyclic olefins with relevant ring-strains. In this so-called ring-opening metathesis polymerization (ROMP, **Scheme 1-2**, d) polymers with molecular weights on the order of 10^5 g mol^{-1} can be obtained. Another metathesis polymerization is the acyclic diene metathesis (ADMET, **Scheme 1-2**, e), an equilibrium step-condensation of dienes. All these transformations have a variety of applications in organic and polymer chemistry and provide access to a broad spectrum of unsaturated compounds.



Scheme 1-2: Common olefin metathesis reactions applied in organic and polymer chemistry.

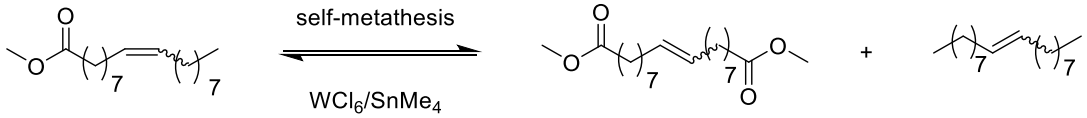
The central role of olefin metathesis is demonstrated by several industrial applications in the petrochemical industry. The first industrial application was the so-called Phillips triolefin process developed at Phillips Petroleum Co. in the 1960s. In this process propylene was converted into ethylene and 2-butene using a heterogeneous WO_3/SiO_2 catalyst system. However, the first plant was shut down already after 6 years due to an increased demand of propylene. Remarkably, running this process in the reverse direction in order to produce propylene became attractive in the subsequent years.[4]

Another prominent example, the Shell Higher Olefin Process (SHOP), was implemented soon after and retains its relevance until today. This large-scale industrial process contains a combination of nickel-catalyzed oligomerization of ethylene, distillation, isomerization by a potassium metal catalyst and metathesis with MoO_3/Al_2O_3 as a catalyst system. In this way linear α-olefins with a chain length between 11 and 14 carbon atoms are produced. These olefins can be converted into aldehydes via hydroformylation and further reduced to detergent alcohols.[4, 5]

1.1.1 Self-Metathesis of Unsaturated Fatty Acids

One promising substrate class for conversion in metathesis reactions are unsaturated fatty acids accessible from renewable resources such as sunflower, palm, or soybean as well as microalgae. Natural oils consist mostly of triglycerides, which are esters derived from glycerol and three (different) fatty acid molecules such as palmitic, oleic or linoleic acid which are some of the most common fatty acids found in nature. Due to their functional groups fatty acids are a

challenging substrate for metathesis and require suitable catalysts which are tolerant against carboxyl groups. The first example was reported in 1972 by Van Dam *et al.* who described the self-metathesis of methyl oleate and methyl elaidate (**Scheme 1-3**) as well as the cross-metathesis of methyl oleate with 3-hexene. In both cases, the rather ill-defined $WCl_6/SnMe_4$ was used as a catalyst system.[6] In this early work thermodynamic equilibrium in the self-metathesis of methyl oleate as well as methyl elaidate was reached within 24 h at 70°C using about 3 mol% of the aforementioned catalyst system. In the cross-metathesis with 3-hexane at 60°C and otherwise similar conditions, a conversion of only 20% was observed after 18 h.



Scheme 1-3: Self- metathesis products of methyl oleate or methyl elaidate.

In 1974, the same group reported the self-metathesis of poly-unsaturated fatty acid esters (linoleic and linolenic ester) with $WCl_6/SnMe_4$ as a catalyst system. After 4 h at 80°C and a catalyst loading of about 3 mol% conversions of up to 95% were reached.[7] Besides alkenes and unsaturated mono- and dicarboxylic esters, the reaction mixtures contained also a considerable amount of 1,4-cyclohexadiene (up to 30% in case of methyl linolenate) which is formed in an intramolecular self-metathesis reaction.

Subsequently, other poorly defined multicomponent catalyst systems for the conversion of unsaturated fatty acids and esters as well as natural oils were developed.[8, 9] However, the conversion of functionalized substrates such as fatty acids using such multicomponent homogeneous and heterogeneous catalyst systems is limited due to their sensitivity to the carboxylic acid or ester groups. The breakthrough came with homogeneous well-defined tungsten and molybdenum complexes developed by Schrock *et al.*[10, 11] and ruthenium based catalysts reported by Grubbs and co-workers.[12] While Schrock carbene metal complexes show high activities and are well-suited for sterically demanding olefins, they are sensitive towards oxygen and moisture. In contrast, ruthenium complexes of the $L_2X_2Ru=CHR$ family are more tolerant towards water and oxygen and therefore show a higher substrate functional group tolerance which is necessary for the conversion of renewable feedstocks.

The most prominent compound of this complex family, the Grubbs 1st generation catalyst (G1, **Figure 1-1**), converts functionalized olefins such as fatty acids successfully. The substitution of one phosphine ligand by a N-heterocyclic carbene leads to the Grubbs 2nd generation catalyst (G2, **Figure 1-1**), which has an increased reactivity and functional group tolerance while maintaining the thermal stability. An even more thermally stable but also more oxygen and moisture tolerant complex was found in the ortho-isopropoxyphenylmethylene chelate. These so called Hoveyda-

Grubbs catalysts (1st and 2nd generation, HG1 and HG2, **Figure 1-1**) show a lower initiation rate though.[13]

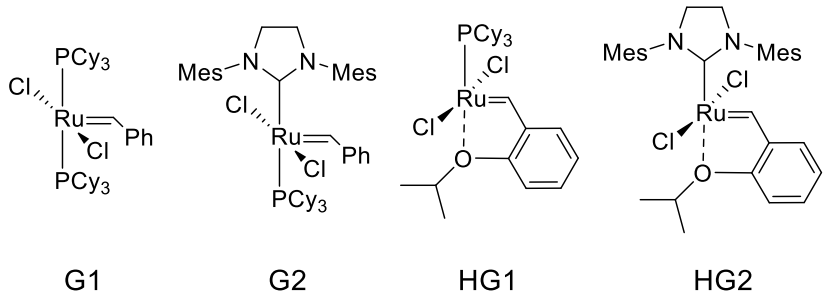
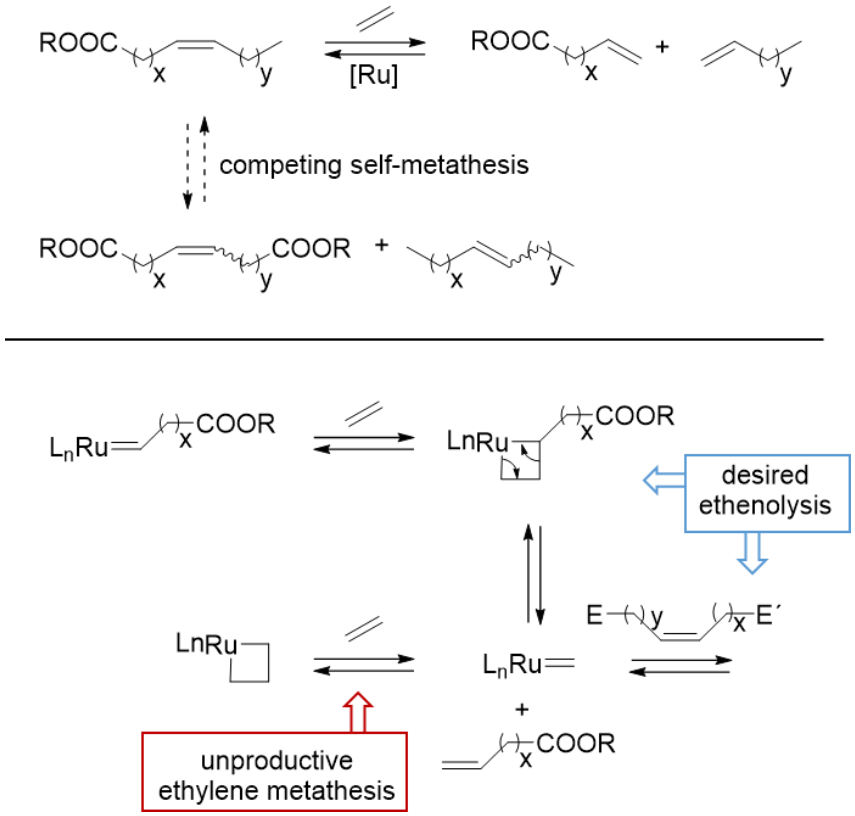


Figure 1-1: Structures of common ruthenium metathesis catalysts. G1: Grubbs 1st generation catalyst, G2: Grubbs 2nd generation catalyst, HG1: Hoveyda-Grubbs 1st generation catalyst, HG2: Hoveyda-Grubbs 2nd generation catalyst.

These well-defined Ru-based catalysts were employed in the self-metathesis of fatty acids and esters as well as in cross-metathesis with various types of olefins such as acrylates, acrylonitriles or ethylene.[14-19] One outstanding example for self-metathesis is the achievement of an effective turnover number (TON) of 440 000 in the self-metathesis of methyl oleate with G2 reported by Dinger *et al*.[19] Nevertheless, molar conversions are limited (max. 50% in homogeneous solution), since such reactions typically are subjected to thermodynamic control. This can only be overcome by shifting the equilibrium for instance by removal of a product.

1.1.2 Cross-Metathesis of Unsaturated Fatty Acids with Short-Chain Alkenes

Besides self-metathesis of unsaturated fatty acids and esters, cross-metathesis with various olefins is a field of current interest. The cross-metathesis with ethylene (ethenolysis) is the perhaps most prominent example. Ethenolysis leads to products with terminal double bonds and in case of the common unsaturated fatty acids found in traditional oil plants to products with an even number of carbon atoms. The terminal products are promising intermediates for the synthesis of fuels, lubricants, waxes or surfactants.[20, 21] A major challenge in the metathesis employing ethylene is to find the trade-off between selectivity and productivity (**Scheme 1-4**). High selectivity for ethenolysis products instead of the competing self-metathesis can be achieved by using high ethylene concentrations. However, higher ethylene concentrations lead to unproductive ethylene self-metathesis and thus have a negative impact on the productivity. Moreover, the formation of unstable methylidene complexes leads to fast decomposition of the catalyst.[22-24]



Scheme 1-4: Ethenolysis of a fatty acid ester (top). Simplified scheme of unproductive ethylene metathesis (bottom). E,E'=alk(en)yl, ester.[25]

Ethenolysis of renewable feedstocks has been reported with well-defined ruthenium as well as molybdenum alkylidenes. Schrock *et al.* reported TONs of up to 5000 in the ethenolysis of methyl oleate using molybdenum complexes.[26] In particular Ru based catalysts have received considerable attention. The first ethenolysis of methyl oleate with commercial available 1st generation Grubbs catalyst was reported by Maughon and coworkers.[22] However, the achieved TON with commercially available Grubbs and Hoveyda-Grubbs catalyst precursors are limited (600-8000)[27] and far away from TONs of >50 000 which has been quoted prerequisite to be of economic interest.[22, 28] However, applying a cyclic alkyl amino carbene complex (CAAC, **Figure 1-2**, left complex) Schrodi *et al.* reached TONs of up to 35 000.[18] This was further improved by Grubbs and co-workers to TONs of 67 000 in the ethenolysis with the same CAAC complex by increasing the purity of the ethylene gas from 99.9% to 99.95%. By reducing the catalyst loading from 10 to 3 ppm they further increased the TON to 120 000. Remarkably, the highest TON (340 000) to date was achieved with 1 ppm of another CAAC ruthenium complex (**Figure 1-2**, right complex) and an even higher ethylene purity (99.995%).

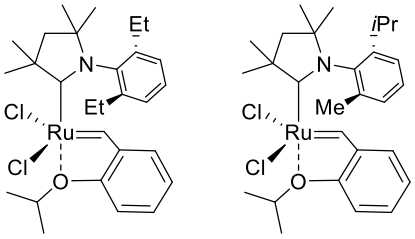


Figure 1-2: Cyclic alkyl amino carbene (CAAC) ruthenium complexes used in the ethenolysis of methyl oleate by Schrodi *et al.* (left) and Grubbs *et al.* (right).

A main limiting factor for using common ruthenium-based catalysts in ethenolysis is the formation of unstable methylidene complexes. Cross-metathesis with internal alkenes can overcome these drawbacks. For the cross-metathesis of synthetic triglyceride of oleic acid as well as natural oils with 2-butene Patel *et al.* reported remarkable TONs between 23 000 and 93 000 at a conversion of over 90% applying HG2 as catalyst.[29] Compared to ethylene, one further advantage of the utilization of 2-butene is the simpler handling, due to its higher boiling point (0.8 to 3.7 °C) it can be easily used as liquid and a higher excesses can be applied more easily to shift the thermodynamic equilibrium. In a further publication the same group reported the successful upscaling of the butenolysis of sunflower oil of up to 300 g oil.[30] In addition, the TONs of butenolysis were improved by using starting materials of higher quality. Using triply distilled methyl oleate and pure *cis*-2-butene (without 1,3-butadiene contamination) with Hoveyda-Grubbs 2nd generation catalyst TONs of up to 470 000 were reached. By addition of 1% of 1,3-butadiene, which leads to complete catalyst deactivation, the negative effect of this substrate was confirmed. This was explained by formation of vinyl-alkylidene ruthenium complexes which are known to be catalytically inactive in metathesis of acyclic olefins.[31]

Cross-metathesis of renewable native triglycerides on an industrial scale was realized in 2013 by Elevance Renewable Science and Wilmar International. The plant in Gresik, Indonesia has a 180 000 metric ton capacity where natural oils such as palm oil are converted in cross-metathesis with 1-butene (**Figure 1-3**). A mixture of unsaturated esters such as 9-decenoic methyl ester and terminal and internal alkenes including decene is obtained which can be further utilized for the production of lubricants and detergents.[25, 32, 33]

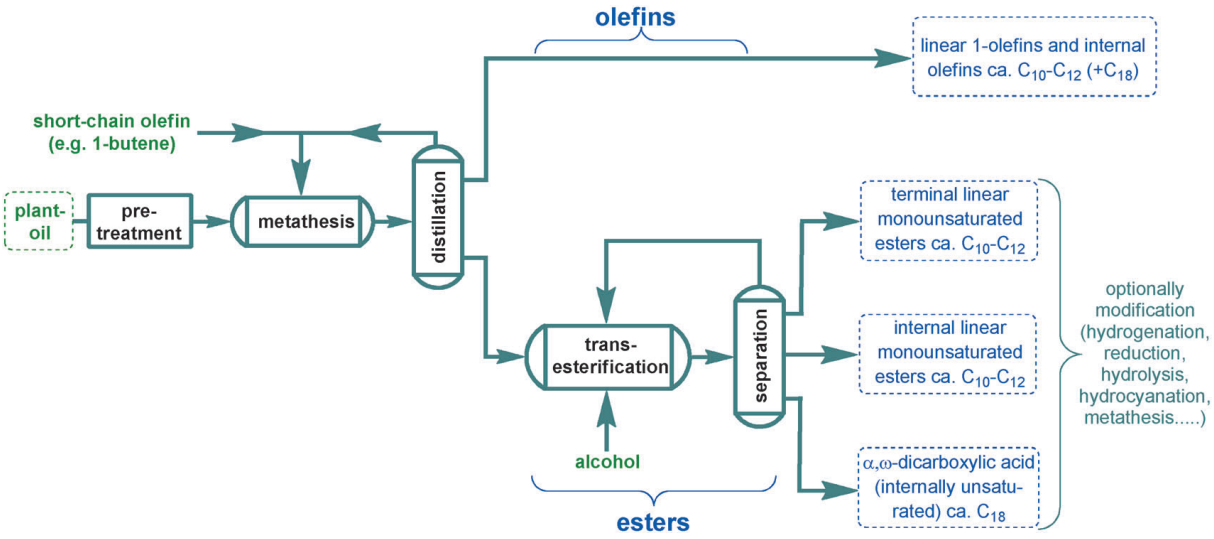


Figure 1-3: Scheme of an alkenolysis process that converts plant-oil triglycerides to medium- and long-chain linear olefins and esters. (Product chain length for C18 feedstock with oleate as a major component.) Reprinted with permission from reference 25. Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

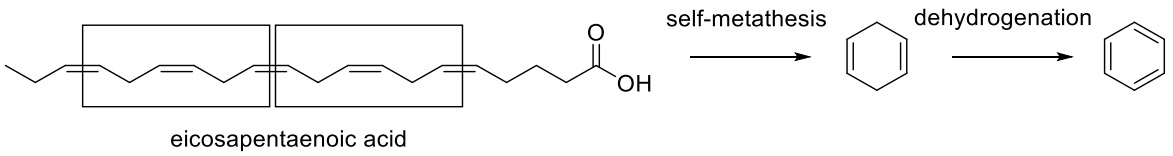
1.1.3 Tandem Reactions of Fatty Acids Involving a Metathesis Step

In general, a tandem reaction is the combination of two or more reaction steps in sequence without isolating the intermediates. Such a simplified reaction process can minimize chemical waste, save time and energy and thus leads to more efficient and environmentally friendly procedures. Yet along with this, the complexity in these transformations in terms of possible desired or undesired pathways is increased. There are different approaches of tandem reactions involving a metathesis step. Most examples in literature couple metathesis reactions with (de)hydrogenation or isomerization.

Gonzales-de-Castro *et al.* described the synthesis of long-chain α,ω-diols from renewable fatty acid methyl esters via the combination of olefin metathesis and subsequent hydrogenation. For this purpose, different Grubbs type catalyst are transformed *in situ* into an active hydrogenation catalyst by adding a base, a bidentate ligand as well as hydrogen. Self-metathesis of methyl oleate forming unsaturated diesters is followed by the hydrogenation of both the double bond and the ester group leading to aliphatic diols. Furthermore, by increasing the molar amount of the bidentate ligand, it is also possible to selectively hydrogenate the ester group. This provides additionally access to unsaturated diols.[34]

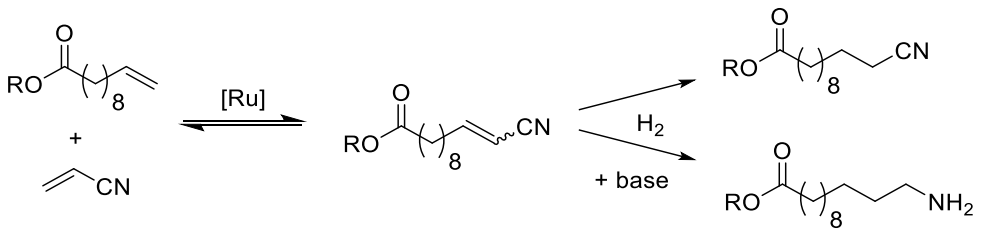
An example for the combination of metathesis and dehydrogenation is the generation of benzene starting from eicosapentaenoic acid, a fivefold unsaturated fatty acid which can be found in various microalgae or yeast. Two equivalents of benzene are effectively produced from one eicosapentaenoic acid molecule in a two-step one pot approach (**Scheme 1-5**). First 1,4-cyclohexadiene is formed in an intramolecular self-metathesis of eicosapentaenoic acid as

demonstrated with various Ru-based metathesis catalysts followed by the dehydrogenation using Pd/C catalysts.[35]



Scheme 1-5: Combination of self-metathesis of eicosapentaenoic acid and subsequent dehydrogenation to benzene.[35]

Malacea *et al.* presented the combination of cross-metathesis and hydrogenation of unsaturated esters providing an access to saturated linear nitrile-acid and -esters as monomers for polyamides (**Scheme 1-6**). [15] They reported the cross-metathesis of terminal and internal unsaturated acid derivates with acrylonitrile as cross-metathesis partner with HG2 as catalyst. The metathesis catalyst was transformed *in situ* into a hydrogenation catalyst by adding H_2 and the crude cross-metathesis mixture was hydrogenated to the desired saturated nitrile esters. By further adding a base (such as *t*BuOK), the unsaturated nitrile esters can also directly be hydrogenated to the corresponding amino ester (**Scheme 1-6**).[36] These α,ω-amino esters formed in a tandem reaction of cross-metathesis and hydrogenation by using a single ruthenium-alkylidene catalyst precursor can serve amongst others as monomers for polyamides.



Scheme 1-6: Cross-metathesis of 10-undecanoic acid ester with acrylonitrile with subsequent hydrogenation to the corresponding nitrile acid ester or amino-acid ester.

Another type of tandem reaction known in literature is the combination of metathesis and isomerization. Generally, in a metathesis reaction, a relevant but in most cases undesirable side reaction is isomerization caused by a ruthenium hydride species, a decomposition product of the Grubbs metathesis catalysts used.[37, 38] Under mild conditions, isomerization occurs in particular with the second generation catalysts. For the first generation ruthenium metathesis catalysts this behavior is only pronounced at elevated temperatures.[39] Although isomerization is often undesired in a metathesis reaction, there are examples in literature in which the isomerization is intended and induced by an additional catalyst. This combination of metathesis and isomerization provides access to a broader product spectrum.

Gooßen *et al.* used a combination of a NHC-indenylidene ruthenium metathesis catalyst and a dimeric palladium(I) complex $[Pd(\mu\text{-}Br)P^tBu_3]_2$ as additional isomerization catalyst to convert oleic acid into an defined distribution of unsaturated compounds (C8-C32 olefins, C13-C25 monocarboxylates and C13-C22 α,ω-dicarboxylates, **Figure 1-4**).[40]

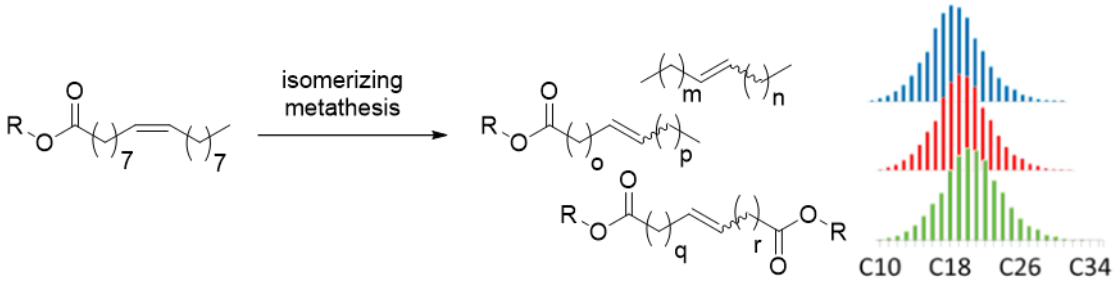
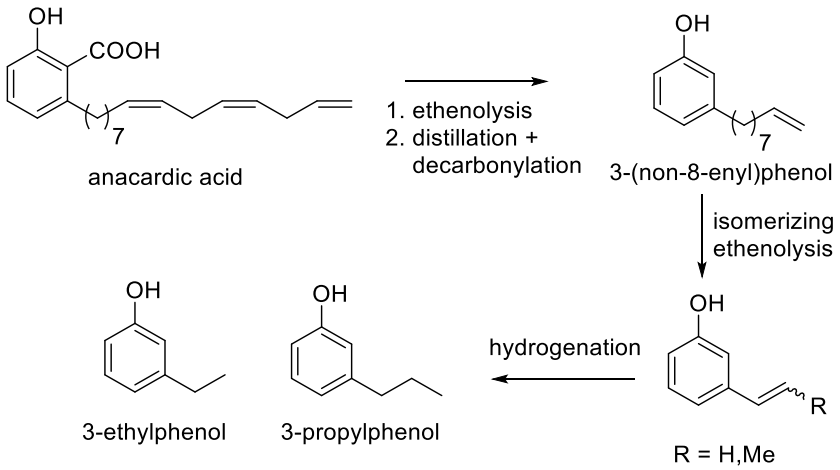


Figure 1-4: Isomerizing self-metathesis of oleic acid derivatives, together with expected product distributions for an ideal catalytic system. Reprinted with permission from reference 40. Copyright 2012 American Chemical Society.

This concept was also applied to the cross-metathesis of oleic acid with different olefinic compounds such as ethylene leading to olefin blends with mean chain length below 18 carbon atoms. By cross-metathesis with *trans*-3-hexenedioic acid a mixture of olefins, monocarboxylates and α,ω-dicarboxylates was obtained. The chain lengths were significantly lower compared to self-metathesis and could be adjusted by the ratio of the starting materials.

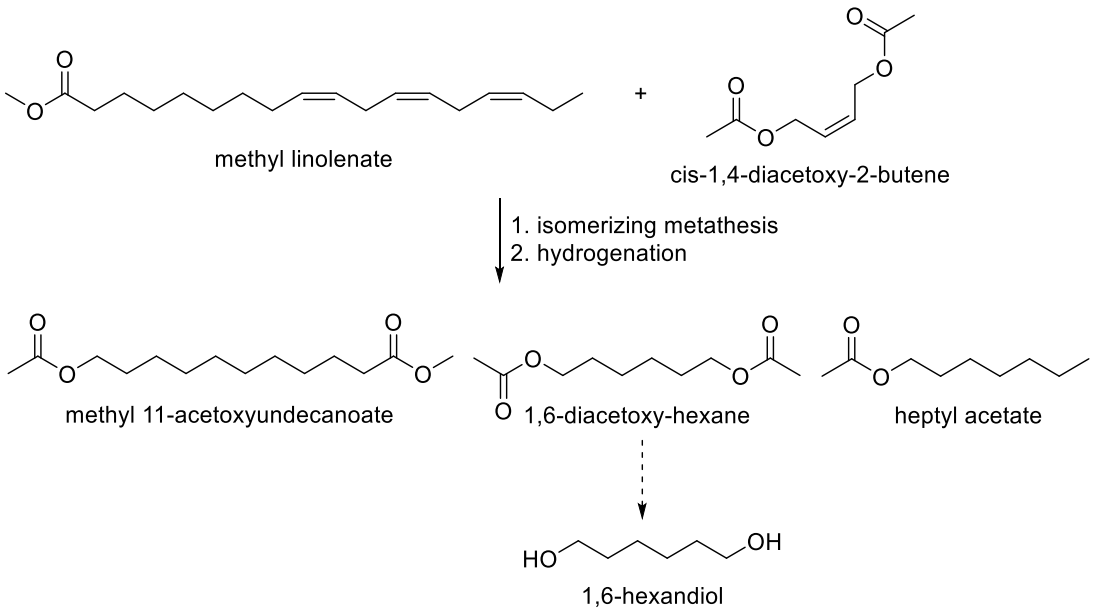
The same group also used isomerizing cross-metathesis for the conversion of rapeseed oil methyl ester and ethylene into biofuel with boiling point specifications described by the fuel standard EN 590.[41] The catalyst system was again $[Pd(\mu\text{-}Br)P^tBu_3]_2$ and Ru metathesis catalysts. The boiling point curve can be influenced by the ratio of ethylene to methyl ester. Additionally, the reaction can be performed under a constant stream of ethylene at atmospheric pressure.

Cole-Hamilton and Gooßen *et al.* also reported the selective synthesis of the tsetse fly attractants 3-ethyl- and 3-propylphenol from a purified cashew nut shell extract containing anacardic acid derivates (**Scheme 1-7**).[42] In a first step they converted the extracted anacardic acid derivates in an ethenolysis reaction with first generation Ru catalysts (without isomerization catalyst and step). In the subsequent distillation the carboxylate group is removed leading to 3-(non-8-enyl)phenol. This is followed by shortening the chain via isomerizing ethenolysis using $[Pd(\mu\text{-}Br)P^tBu_3]_2$ as additional isomerization catalyst. The last step of the synthesis is the hydrogenation leading to the tsetse fly attractants 3-ethyl- and 3-propylphenol (**Scheme 1-7**).



Scheme 1-7: Synthesis route of 3-ethylphenol and 3-propylphenol from anacardic acid including a isomerizing ethenolysis step.

Recently, Tuba and co-workers combined isomerization, metathesis and hydrogenation and presented the synthesis of 1,6-hexandiol.[43] Methyl 11-acetoxyundecanoate, heptyl acetate and 1,6-diacetoxy-hexane, that can be used as a precursor of 1,6-hexandiol, were obtained in 53-99% yield via a one-pot isomerization metathesis of α-linolenic acid methyl ester and *cis*-1,4-diacetoxy-2-butene with Grubbs metathesis catalysts (3 mol%) and [$RuHCl(CO)(PPh_3)_3$] (2 mol%) as isomerization catalyst followed by Pd/C catalyzed hydrogenation (**Scheme 1-8**).



Scheme 1-8: Synthesis route of 1,6-hexandiol from methyl linoleate including isomerizing metathesis.

Apart from the combination of metathesis and hydrogenation or isomerization, Patel *et al.* reported a one-pot metathesis-isomerization-methoxycarbonylation-transesterification reaction sequence producing terminal oxygenates from natural oils such as sunflower or linseed oil. The

butenolysis with HG2 as catalyst was terminated with ethyl vinyl ether once high conversion (up to 99%) has been achieved. Then bis(di-tert-butylphosphino)xylene, bis(dibenzylidene-acetone)palladium(0), methanesulfonic acid, 28 bar CO and methanol was added for the isomerizing methoxycarbonylation. They obtained mixtures of esters (C6, C9 and C12) and diesters (C9, C12) with selectivities for terminal esters of over 95%.[44]

1.2 Microalgae as a Source of Lipids

In nature, several microalgae strains produce significant amount of lipids which fulfil different functions: On the one hand, there are membrane lipids enclosing the cell, on the other hand lipids can serve as energy storage.[45, 46] Microalgae oil consists of a unique composition of fatty acids with unusual chain length (e. g. palmitoleic acid) and high amounts of (poly-) unsaturated fatty acids such as eicosapentaenoic acid or docosahexaenoic acid barely found in traditional plant oils (e.g. sunflower, rapeseed or soybean). Moreover, microalgae as renewable source for lipids also have other potential advantages over common oil plants. They can be grown in brackish or salt water and do not need arable land, thus microalgae do not compete with food production.[47] Furthermore, a special property of microalgae are their high growth rates. They can double their biomass within 24 hours.[48] In addition, their lipid content of up to 70% of dry weight is remarkable.[49]

Today, the most prominent fatty acids produced from microalgae are the poly-unsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid. These ω-3 fatty acids extracted from microalgae are commercially available as human health supplements.[50] Furthermore, the application of microalgae lipids for the production of biofuel has gained much attention in research and politics since the 1950s.[51] Yet, due to limitation of algae growth and the energy-intensive downstream processes, such as harvesting and extraction, biofuels based on microalgae lipids are not commercially viable.[50, 52]

Also, the application for biofuels appears less sensible in that it seeks to defunctionalize the fatty acid substrate. Current approaches for utilization of microalgae oil focus on high-value chemicals. With their long and unique hydrocarbon chain, the carboxylic acid and in case of unsaturated fatty acids the double bond(s), microalgae fatty acids in general are attractive for catalytic valorization to access chemical intermediates and allow for much broader applications.

1.2.1 *Phaeodactylum tricornutum*

Phaeodactylum tricornutum (*P. tricornutum*) is a eukaryotic microalgae and assigned to the class of diatoms. This marine microalgae differs from other diatoms: It appears in four different morphotypes: fusiform, triradiate, oval[53] and cruciform (**Figure 1-5**).[54] The reason for this is its low silica content in the cell wall compared to other diatoms.[55]

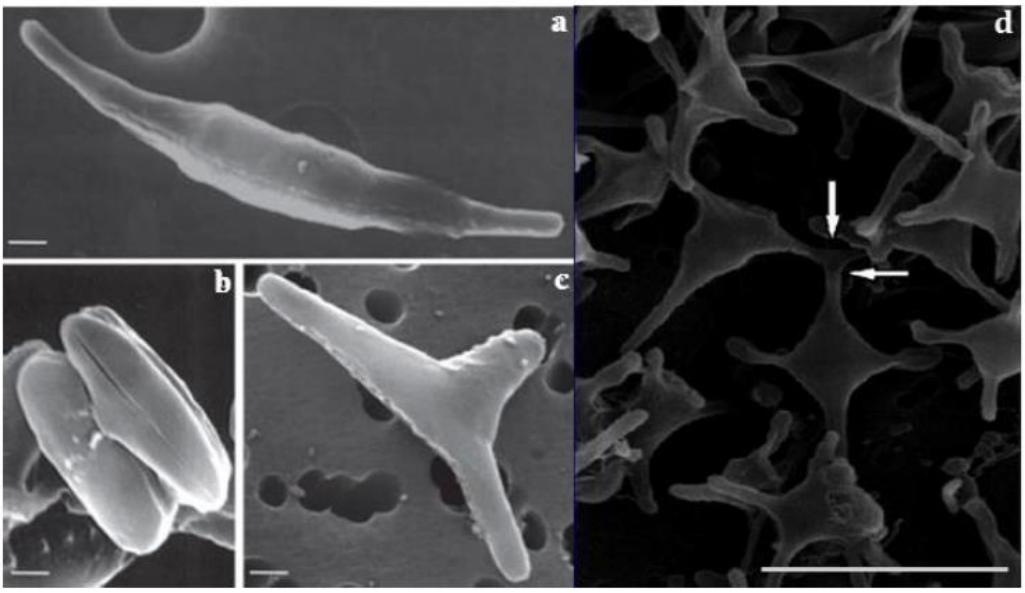


Figure 1-5: Different morphotypes of *Phaeodactylum tricornutum* in SEM images. a – fusiform; b – oval; c – triradiate; d – cruciform; scale bar for a – c: 1 µm; scale bar for d: 10 µm. Reprinted with permission from reference 54, 55. Copyright 2014 He *et al*. and 2007 Phycological Society of America.

P. tricornutum is one of the main diatom model organisms. The reasons for this are the flexibility in their growth conditions, a short doubling time and its photoautotrophic behaviour. Furthermore, it shows a high unsaturated fatty acid content, which was a decisive aspect to use this microalgae strain in this work. The fatty acid composition is shown in **Table 1-1**.

Table 1-1: Composition of fatty acids extracted from *Phaeodactylum tricornutum* in the exponential and stationary phase of its growth cycle determined by Tono *et al*.[56]

Fatty acid	**Exponential phase [mol%]**		**Stationary phase [mol%]**	
Myristic acid	11.12	±0.34	8.7	±0.17
Palmitic acid	13.41	±0.05	25.34	±0.33
Palmitoleic acid	29.30	±0.09	42.92	±1.03
Stearic acid	0.69	±0.04	0.68	±0.07
Oleic acid	5.31	±0.04	4.34	±0.12
Eicosapentaenoic acid	29.98	±0.10	11.57	±0.58
Docosahexaenoic acid	3.10	±0.02	1.00	±0.04

The fatty acid composition and production can be influenced by the cultivation parameters such as light conditions, temperatures, pH value, CO_2 concentration and nutrient supply. Furthermore, complete sequencing of the genome of *P. tricornutum*[57] opened up the possibility for genetic modifications that result in variations in the fatty acid content and composition.[58-61]

1.2.2 Laboratory Scale Cultivation and Lipid Extraction with Organic Solvents

Cultivation of microalgae can be performed in continuous or batch operations. In large scale they are grown in open ponds[62] or photobioreactors.[52] On a laboratory scale, batch-wise cultivation in flasks can be employed as a simple and flexible procedure. The bottleneck of the oil production from microalgae in relation to cost and energy consumption is the isolation of the algae and extraction of the desired compounds. In autotrophic algae culture the cell concentration with 0.1-8 g dry weight L^{-1} is low,[63] and a large amount of water has to be separated in the downstream processing. Another disadvantage for harvesting is the small size of the microalgae cells (diameter of 5-50 μm)[64] and cells need to be harvested by centrifugation or filtration.

Lipid extraction can be achieved with an organic solvent system or using supercritical fluids such as CO_2. The latter is discussed in detail in chapter 1.3.1. Since microalgae, especially diatoms, have thick cell walls and the algae store their lipids in the cytoplasm, a previous pre-treatment for cell disruption such as autoclaving, bead-beating, ultrasonication or microwave can improve the efficiency of the extraction.[49, 65-68]

The most prominent organic lipid extraction methods in literature were described by Folch *et al.* in 1951 and Bligh and Dyer in 1959.[69-71] Both methods were originally developed for the extraction of lipids from brain or fish tissue for analytical purpose and employ the same biphasic solvent system of $CHCl_3$/MeOH/H_2O. As water is added intentionally, drying of algae is not necessary and reduces the steps in the downstream processing. The chloroform phase of this solvent system contains the full range of lipids whereas the proteins and carbohydrates are enriched in the water phase.

Bligh and Dyer improved the Folch method by changing the solvent ratio (Folch 8:4:3; Bligh and Dyer 2:2:1.8) as well as decreasing the amount of solvent per gram sample (solvent sample ratio 20:1 and 4:1, respectively). However, it was found, that the Bligh and Dyer method is not applicable for samples containing more than 2 wt% oil. In this case the lipid extraction is not complete and the oil content is underestimated by up to 50%.[72] This is the reason why the Bligh and Dyer method is not suitable for lipid extraction from microalgae algae as they contain up to 70% lipids of their dry weight.

Several attempts were made to find less toxic solvent systems as alternative to the Folch method.[68-70] However, no suitable alternative was found so far, and the most efficient method is still the one described by Folch *et al*.[49, 73-75] Note that in technical processes, hexane is used as a solvent to extract microalgae lipids.[46]

1.2.3 Chemical Valorization of Microalgae Lipids

In literature there are only a few examples of the utilization of the unique feedstock of fatty acids extracted from microalgae as starting material for chemical functionalization.

Our group reported the catalytic upgrading of algae oil extracted via the Folch method from the microalgae strain *P. tricornutum.* Via isomerizing alkoxycarbonylation with methanol and [Pd(dtbpx)(OTf)$_2$] as catalyst precursor the mono-unsaturated fatty acids of the crude oil were selectively converted into α,ω-diesters whereas the alkoxycarbonylation of the poly-unsaturated fatty acid, eicosapentaenoic acid, gave a broad unselective product spectrum. Remarkably, the catalyst was still active in the presence of various components of the crude oil such as carotenoids, chlorophylls, carbohydrates or phosphocholines. Subsequently, the diester products from the isomerizing alkoxycarbonylation were successfully used in a polycondensation reaction leading to a long-chain aliphatic polyester based on fatty acids from microalgae.[68]

As the 5-fold unsaturated fatty acid eicosapentaenoic acid was not selectively converted to the linear diester in the isomerizing alkoxycarbonylation a selectively hydrogenation to the mono-unsaturated fatty acid was investigated. The resulting mono-unsaturated compound was subsequently transformed into an α,ω-diester via isomerizing alkoxycarbonylation.[76] This dual approach with selective heterogeneous hydrogenation and homogeneous carbonylation is also suitable for tall oil and crude microalgae oil containing mono- and polyunsaturated fatty acids. The algae oil of genetically engineered *P. tricornutum* with an enhanced eicosapentaenoic acid content (ca. 50% of the unsaturated fatty acids) was used and quantitatively converted to the linear α,ω-diester with an overall selectivity of 75%.

Another example for the valorization of microalgae is direct catalytic upgrading of wet algae biomass via CO-free alkoxycarbonylation.[77] By using methanol and formates instead of carbon monoxide, the unsaturated fatty acids were simultaneously extracted and converted to linear long-chain diesters without additional workup and extraction steps. This was possible with high selectivities to the linear diester over 90%, which can serve as building blocks for renewable polyesters as shown in the example before.[68]

Ethenolysis of microalgae and yeast oil for production of biofuels and chemicals was reported by Chuck *et al*.[78] The cross-metathesis of microalgae oil from *Pseudochorisystis ellipsoidea* with ethylene with a ruthenium alkylidene catalyst occurred with a selectivity of 35-40% for terminal double bonds while a reaction with oil from *Scenedesmus obliquus* did not yield any terminal double bonds.

1.3 Supercritical Carbon Dioxide as Reaction and Extraction Medium

Supercritical fluids have gained much interest and technological relevance since the first reports of critical points by Cagnirad de La Tour in 1822.[79] In his experiments he demonstrated the existence of a limiting temperature beyond which no liquefaction via further compression is possible and no phase interface exists. The supercritical state of CO_2 was reported 47 years later.[80] With a critical temperature of 31.0 °C and critical pressure of 73.8 bar (density of 0.477 g mL^{-1}, see **Figure 1-6**[81]) CO_2 has mild critical parameters. Beyond this point, neither a liquid nor a gas phase is present. The supercritical phase has unique properties, which are reminiscent of both the liquid and gas phase. $ScCO_2$ has a liquid-like density and can dissolve substances like a liquid. Besides this, it can diffuse through porous solids like a gas.

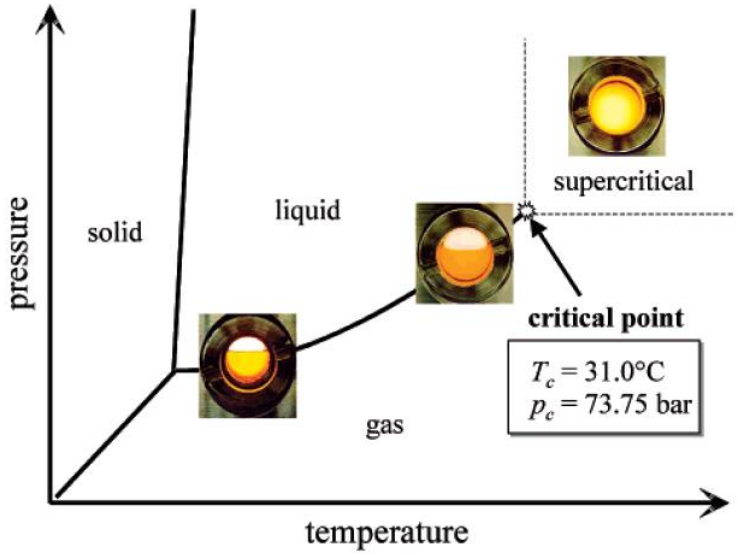


Figure 1-6: Schematic phase diagram of CO_2 with snapshots of the transition from the liquid/gas region to the supercritical region (a bright orange "CO_2-philic" rhodium complex was added for better contrast). Reprinted with permission from reference [81]. Copyright 2002 American Chemical Society.

In general, CO_2 is relatively cheap, non-toxic and environmentally friendly. Furthermore, it has a good heat transport capacity and is inert to a wide range of chemicals. Due to the linear structure of the CO_2 molecules it has an apolar character. By varying pressure or temperature, the density and thus the solvent properties can be tuned. All this makes $scCO_2$ a green alternative to organic solvents.

In particular, the mild critical parameters which allow for the extraction of thermal sensible compounds as well as the possibility to vary the solvent properties easily and thus the selectivity make $scCO_2$ an attractive extraction solvent. One further advantage is the fast penetration of the

biomass due to its supercritical properties, leading to short extraction times and high yields. Last but not least, CO_2 is easily removed after extraction and does not remain in the product.[49] Yet, compression of the fluid at elevated temperature makes it quite energy-intensive. Nevertheless, the broadest application of $scCO_2$ as a solvent is in extraction. Currently, it is applied in the food and nutrition industry[82] for the decaffination[83] of coffee or the extraction of astaxanthin[84] from microalgae. In recent years supercritical fluid extraction of lipids, mainly ω3-fatty, acids have gained importance due to their nutritional and health applications.

Besides the utilization as extraction medium, $scCO_2$ has been reported as solvent in many chemical reactions such as hydrogenation,[85, 86] hydroformylation,[87] oxidation,[88] esterification,[89] Diels-Alder reaction,[90] polymerizations[91] and metathesis.[92, 93] Thereby often the low environmental impact of this green solvent is highlighted. However, one further advantage is that the interaction with the catalyst and/or the substrate can be influenced by adjusting the density, and thus the solvent properties. Using this correlation, the activity and selectivity can be controlled. Moreover, another advantage of using $scCO_2$ as reaction medium is a possible simplification in workup.[81, 94] Limitations of the utilization of $scCO_2$ as reaction medium include the low solubility of polar substances and high costs and effort due to high operating pressures. Furthermore, $scCO_2$ may take part in the reaction, which might not be desired.

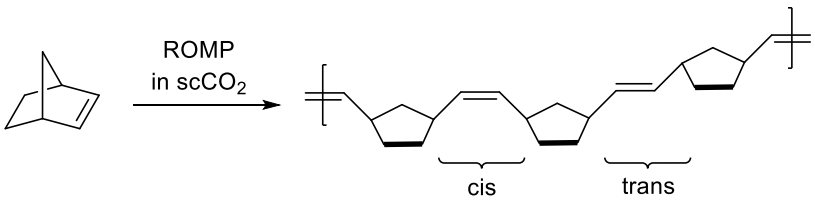
1.3.1 $ScCO_2$ as Extraction Medium for Microalgae Oil

Due to its apolar character, $scCO_2$ is suitable for the extraction of apolar lipids (mainly triglycerides) from various biomasses such as plants, animals or microorganism. The topic of lipid extraction of microalgae is covered in many articles and reviews,[82, 95-105] many of them with regard to biofuel production. However, the reported optimized parameters are often different, and the results are sometimes inconsistent. In general, higher pressure has a beneficial effect on the extraction yield as the solubility of lipids increases with pressure. In contrast to this, the effect of temperature is more complex. At low pressure an increase in temperature has a negative effect, whereas at high pressures a positive effect of increasing temperature can be observed.[106] Furthermore, there are many other aspects that influence the extraction such as duration of extraction and the fluid flow rate. Additionally, optional cell disruption pre-treatment as well as the remaining water content affect the extraction yield. Furthermore, additional co-solvents such as ethanol or methanol are decisive to the extraction result and can help to extract also polar substrates. Besides this, different extractor set-ups as well as different algae strains and cultivation conditions have an influence on the extraction outcome. As a consequent of all this, comparisons of the results of the different references are quite difficult.

In many literature reports the microalgae strains *Spirulina platensis* and *Chlorella vulgaris* are used.[106] Despite the advantages described in chapter 1.2.1 of the herein used *P. tricornutum*, only a few groups reports $scCO_2$ extraction of this particular microalgae strain. For example, Tommasi *et al.* described the extraction of lipids from the microalga *P. tricornutum* by dimethyl carbonate and $scCO_2$ using deep eutectic solvents and microwave heating as pre-treatment.[101] Their work focusses on the pre-treatment of the biomass, keeping the CO_2 extraction conditions constant (350 bar, 45 °C, ρ=0.92 g mL^{-1}, flow rate 2.5 L min^{-1}, 25 min static extraction, 100 min dynamic extraction). It was demonstrated that the extraction efficiency can be significantly improved from 1 to 7 wt% of the dry weight by pre-treatment with deep eutectic solvents and microwave to destroy the thick silica wall of the diatom. In contrast, with Bligh and Dyer organic solvents extraction a significant higher yield (31 wt%) was obtained. However, the purity of the obtained crude oils differs significantly. While the $scCO_2$ extracted oil contains almost pure fatty acids (as triglyceride ester), the organic solvent extraction only shows a selectivity towards fatty acids of about 35%. This illustrates the high selectivity for apolar triglycerides in $scCO_2$ extraction leading to oils with high purity. All in all, CO_2 is attractive for the selective extraction of triglycerides from microalgae.

1.3.2 $ScCO_2$ as Reaction Medium for Olefin Metathesis

For metathesis reactions with $scCO_2$ as a reaction medium only a few examples have been reported so far. A first publication addresses ring-opening metathesis polymerization (ROMP) of norbornene at 45 °C and CO_2 pressures between 60 and 345 bar with $[Ru(H_2O)_6(tos)_2]$ as olefin metathesis catalyst precursor which is insoluble in the reaction medium (**Scheme 1-9**).[92] Yields and molecular weight typically on the order of several 10^4 g mol^{-1} of the obtained poly(norbornene) were comparable to those synthesized in conventional organic solvents. Addition of different amounts of methanol to improve the initiator solubility has an influence on the *cis/trans* ratio of the polymer microstructure. Further investigations showed, that under high pressures of pure CO_2 a syndiotactic poly(norbornene) with high *cis*-stereoselectivity was formed.[92, 107]

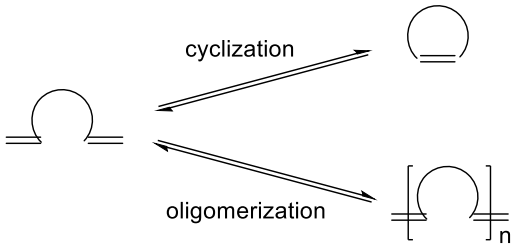


Scheme 1-9: Ring-opening metathesis of norbornene.

Compared to the use of $[Ru(H_2O)(OTos)_2]$ as catalyst precursor, Fürstner *et al*. reported higher activities with well-defined Ru alkylidene catalysts (Grubbs 1st and 2nd generation type, see also **Figure 1-1**) in ROMP of norbornene and cyclooctene, respectively. The reactions were conducted in both liquid and supercritical CO_2 at temperatures between 25 and 57 °C and CO_2 pressures of 56 and115 bar.[93, 108] The properties of the obtained polymer are comparable to those formed in dichloromethane. Under optimized conditions for ROMP of norbornene at 37 °C and 165 bar, a yield of 97% with a catalyst loading of 0.02 mol% was obtained. ROMP of norbornene with a molybdenum alkylidene catalyst (0.16 mol%) was successfully performed. While the Ru-catalyst is distributed heterogeneously, the molybdenum complex is dissolved in CO_2.[93, 108]

Furthermore, Cassidy and co-workers investigated the ROMP of norbornene in $scCO_2$ (70 °C and 207 bar) using different Grubbs and Schrock type catalysts.[109] The obtained polynorbornenes show similar molecular weights, molecular weight distributions and thermal stability compared to those prepared in THF. With the Grubbs catalyst, a high *trans*/*cis* ratio is obtained, whereas with the Schrock catalyst the ratio was reversed. Furthermore, by adding different amount of co-solvent (THF, toluene, DMF, DMSO) and varying the catalyst concentration, *trans/cis* ratios between 3.2:1 and 5.5:1 can be adjusted.

In addition to ROMP, Fürstner *et al.* also reported ring-closing-metathesis (RCM) forming products of different ring-sizes in CO_2 with Grubbs 1st and 2nd catalyst as well as a Schrock molybdenum catalyst.[93, 108] By variation of the CO_2 density the competition between intra- and intermolecular reaction (cyclization and oligomerization, **Scheme 1-10**) can be influenced. At high CO_2 densities, cyclization is favoured while at lower densities, which means a higher local concentration of the diene, oligomerization is preferred. Thereby also enyne RCM was successfully performed in $scCO_2$.[93]



Scheme 1-10: The competition between intra- and intermolecular reaction in the metathesis of dienes.

A further advantage of CO_2 as reaction medium is the acido-basic properties of CO_2. Grubbs 1st generation catalysts are known to be deactivated in organic solvents by free N-H groups. However, in $scCO_2$ it was observed that dienes containing this functionality react without the need of additional protecting groups. CO_2 forms the corresponding carbamic acids and thus protects

the amino group. This was demonstrated by the RCM of the compound shown in **Figure 1-7**, which does not take place in dichloromethane as a reaction medium in contrast to RCM in CO_2.[93]

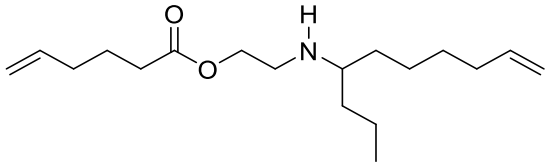
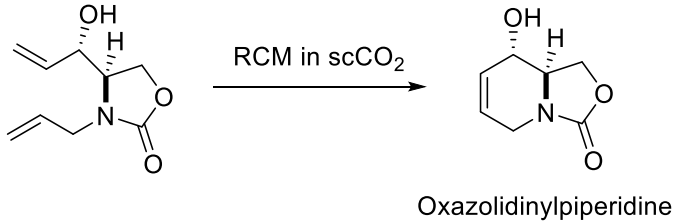


Figure 1-7: Structure of the diene used in RCM in $scCO_2$.

Another example for the synthesis of small molecules synthesis via metathesis in $scCO_2$ is the preparation of oxazolidinylpiperidine (**Scheme 1-11**). This precursor of glycosidase inhibitor was prepared via Ring closing metathesis in $scCO_2$. At a CO_2 pressure of 200 bar and 40 °C applying 2 mol% Grubbs catalyst a yield of 88% is obtained.[110]



Scheme 1-11: Preparation of oxazolidinylpiperidine by ring-closing-metathesis (RCM) in $scCO_2$.

Furthermore, covalently immobilized Hoveyda-Grubbs type catalyst was active in a RCM in $scCO_2$ as a reaction medium for an array of different starting materials.[111] The experiments were carried out at 40 °C and 140 bar CO_2 with a catalyst loading of 2.5 mol%. The performance depends on the support material of the catalyst but was in all cases lower than the non-immobilized catalyst. Notably, low leaching (20 ppm) of ruthenium into the product was observed.

Another example of heterogeneously catalyzed metathesis in $scCO_2$ is the self-metathesis of α-olefins (C6-C8) at 35 °C and 80-150 bar CO_2.[112, 113] The reaction was catalyzed by supported Re-oxide Re_2O_7 (7%) and conversions over 30% higher than in conventional solvents such as toluene or *n*-heptane were obtained. This observation is explained by the increase of the mass transfer promoted by dense CO_2. Yet the conversion strongly depends on the support material. In the self-metathesis of 1-octene for Re_2O_7 supported on γ-Al_2O_3 high conversions of up to 82% were found, whereas no conversion for silica-based systems was observed.

This approach provides the opportunity for metathesis under continuous-flow conditions with $scCO_2$ as carrier.[114] For the self-metathesis of 1-octene catalyzed by Re_2O_7 supported on γ-Al_2O_3 a continuous-flow procedure was reported. The best results were achieved at 100 °C and 90 bar, operating at flow rates of 0.05 and 1 mL min^{-1} for 1-octene and $scCO_2$, respectively. High

selectivities (>90%) for the self-metathesis products and an average productivity of 0.24 mL tetradecene $g_{Re}^{-1}min^{-1}$ were observed. It is notable that $scCO_2$ increased the catalyst lifetime and improved and restored its performance compared to conventional organic solvents such as toluene or *n*-hexane. This allows efficient recycling. The reversibility of the deactivation of Re-oxid in the presence of $scCO_2$ was demonstrated.

Furthermore, Yang *et al*. investigated the phase behaviour of the ethenolysis of ethyl oleate in compressed CO_2 (50-120 bar, 35-50 °C).[115] They demonstrated that CO_2 at suitable pressures and temperatures can enhance the reaction rate and conversion. With an ethylene pressure of 15 bar and at a total pressure of 82 bar at 35 °C, applying 1 mol% of Grubbs 1st generation catalyst conversions of up to 90% were achieved. Note that under these conditions a liquid as well as gas phase is existent which is essential for such high reaction rates which can be explained by different solubilities. The solubility of the ethenolysis products in the gas phase is high, whereas the solubility of ethyl oleate in the gas phase is low. As a consequence, the formed products are extracted into the gas phase and the equilibrium is shifted. At 120 bar all substrates including the products are soluble in the supercritical fluid phase, but the catalyst was not well dispersed leading to lower conversions.

In addition, $scCO_2$ was used for the removal of ruthenium catalyst (and its by-products) from a crude RCM reaction mixture. The high solubility of the formed macrocycle enables the separation from the catalyst which remains in the autoclave. In addition, the extraction in a continuous mode was evaluated. For this purpose a crude mixture of the macrocycle in toluene containing 5 mol% Hoveyda-Grubbs 1st generation catalyst was used leading to 88% of the isolated macrocycle and a level of ruthenium of 700-800 ppm.[116]

All in all, as the catalyst activity is not negatively affected, $scCO_2$ is a suitable reaction medium for olefin metathesis and a promising alternative to organic solvents. Achieving a sufficient solubility of molecular catalysts, or reactivity of heterogeneous catalysts, can be a challenge. Moreover, there are also some examples in literature in which $scCO_2$ has a beneficial effect on activity and selectivity. Additionally, as solvent residues in the reaction products are not an issue, the workup and isolation are highly simplified making $scCO_2$ an environmentally benign reaction medium fur sustainable chemical synthesis.

2 Scope of the Thesis

Recent studies on the utilization of microalgae oil as a feedstock have largely focussed on the production of biofuel. However, this does not exploit the full potential of the unique structure of fatty acids present in microalgae with up to 70% of their dry weight. The carboxyl functionality and in case of (poly-)unsaturated fatty acids the double bond(s) are not utilized in case of biofuel production. However, these functional groups make fatty acids an attractive substrate for catalytic functionalization such as olefin metathesis or carbonylation and thus for the valorization to various chemical intermediates. Furthermore, algae oil contains a unique composition of fatty acids with unusual chain lengths and a high share of (poly-) unsaturated fatty acids which are not found in traditional plant oils leading to a broader product spectrum accessible via catalytic valorization.

The scope of this thesis comprises the utilization of unsaturated fatty acids extracted from the microalgae *Phaeodactylum tricornutum* for the selective upgrading to higher value chemicals via olefin metathesis and subsequent catalytic transformations. Based on self- and cross-metathesis, in particular ethenolysis or butenolysis, and further catalytic transformations such as isomerizing alkoxycarbonylation, a range of different products can be targeted, some of which currently only accessible via demanding synthetic routes. One challenge is the potential deactivation of the catalysts applied by the various components in the crude oil such as carotenoids, chlorophylls, carbohydrates or phosphocholines. Furthermore, unwanted side reactions such as isomerization or in case of cross-metathesis self-metathesis have to be supressed to a sufficient extent.

The utilization of microalgae as biomass has a significant bottleneck; the workup and the extraction of the oil often requires energy-intensive multi-step procedures and the use of organic and halogenated solvents which are environmentally harmful. To simplify the overall process the extraction and the following chemical conversion should be adapted to each other. For this purpose, $scCO_2$ is a suitable extraction and reaction medium, which is relatively cheap, non-toxic, inert and environmentally friendly. To this end, extraction of microalgae lipids with supercritical CO_2 is investigated and optimized for the particular feedstock and technical conditions employed. Alongside, the viability of catalytic olefin metathesis of the lipid mixture in dense CO_2 is explored. Ultimately, both steps will be combined and applied to the crude biomass to demonstrate a biorefinery concept for the synthesis of higher value chemicals based on microalgae lipids.

3 Catalytic Refining of Microalgae Oil via Butenolysis and Carbonylation in Organic Solvents

3.1 Introduction

Due to their unique molecular structure, fatty acids in general are attractive substrates that offer themselves for further functionalization of the carboxylic acid ester group or transformation of the (unsaturated) alkyl chain.[117, 118] This applies in particular to fatty acids present in algae oil, which consists of a unique composition. Unusual chain lengths and high amounts of poly-unsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid are relatively abundant in algae oil, whereas these are barely found in traditional seed oils. Algae oils have received much attention as a new biomass source to produce renewable energy in the form of biodiesel. Approaches such as transesterification or deoxygenation/hydrocracking have been employed to obtain suitable material for fuel. These aim at mimicking petroleum-based hydrocarbon fuels. However, they do not exploit the full potential of these algal fatty acids in making use of its functionality and possibility for further functionalization.[46]

So far predominately fatty acids from seed oils such as sunflower, rapeseed and palm oil are being used for the production of chemicals or monomers for polymers.[25, 118] However, growing plants for the oil production raises issues such as occupation of arable land and consumption of irrigation water, a competition with food production, inefficient yields per time and area, and the associated logistics of harvesting and collection. In contrast, algae can be cultivated in brackish or saltwater on non-arable land that is unapt for food production. Moreover, the oil production of microalgae can be much higher compared to traditional crops.[48, 50] Short-chain dicarboxylic acids (up to C6) are produced on a large scale by petrochemical routes, and increasingly also by fermentation of carbohydrate feedstocks. By contrast, mid-chain (C7 – C12) dicarboxylic acids are most commonly generated from seed oil fatty acids. Some of these dicarboxylic acids are only accessible via tedious synthetic routes. Azelaic acid (1,9-nonanedioic acid), for example, is

produced on an industrial scale via ozonolysis of oleic acid on a scale of several 1000 tons per year.[119] Ozonolysis is a technical challenging and potentially hazardous process, however.

An attractive concept of an algae-based refinery to produce mid-chain olefins and carboxylic acids or esters is the utilization of the double bond of unsaturated fatty acids in a cross-metathesis reaction. Moreover, by employing short-chain olefins, e.g. ethylene or butene, in the cross-metathesis with unsaturated fatty acids, the chain length of the products can be modified and thus expands the range of desirable products accessible by this algae-based refinery approach. An approach to use microbial and yeast strains for production of biofuels by metathesis was reported by Chuck *et al*.[78] Ethenolysis of microalgae oil from *Pseudochorisystis ellipsoidea* occurred with a selectivity of 35-40 % towards terminal double bonds while for oil from *Scenedesmus obliquus* only cross- and self-metathesis products of the fatty acids were found, and no terminal double bonds were obtained. During ethenolysis, an unstable methylidene intermediate catalyst species is formed, which can lead to rapid decomposition of the catalyst. This results in limited productivity. In cross-metathesis with internal olefins, no such methylidene intermediate is formed.[22-24] As products internal olefins are obtained. Butenolysis of seed oils such as sunflower, canola, soya and linseed was demonstrated by Robinson[29, 30] along with the subsequent alkoxy-carbonylation of those mixtures.[44] Butenolysis of neat commercially available methyl oleate occurred with a total of 1800 turnovers. Concerning industrial applicability of olefin metathesis, it is notable that butenolysis of palm oil is applied on an industrial scale in the Elevance Biorefinery process.[25, 120] In this chapter a two-step fully catalytic route towards value-added products from microalgae oil is presented (**Figure 3-1**).

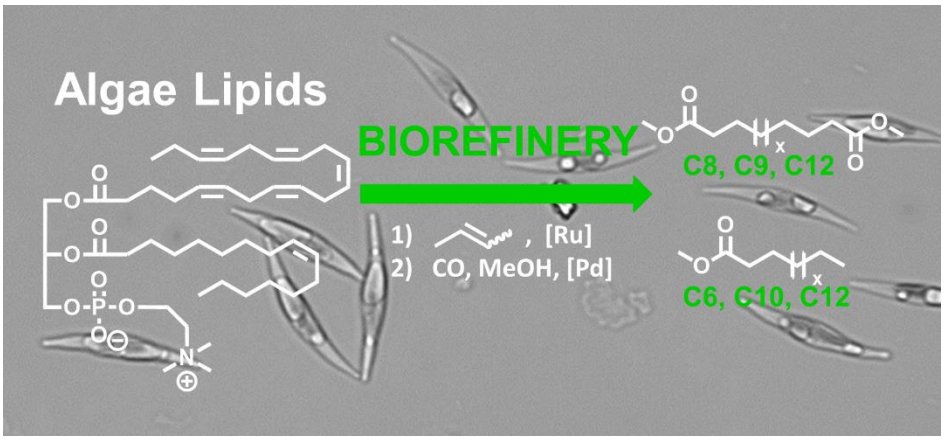


Figure 3-1: Schematic representation of the two-step fully catalytic route towards mid-chain carboxylic (di)acid esters from microalgae oil.

3.2 Results and Discussion

3.2.1 Algae Oil from *Phaeodactylum tricornutum*

The microalgae strain *Phaeodactylum tricornutum*, a unicellular diatom, was used as a lipid source, as it produces relatively large amounts of unsaturated fatty acids.[56] The algae oil was extracted by a modified Folch method[69](c.f. chapter 3.4.2 Algae Cultivation and Extraction). In this way from 30 L algae culture, being in the stationary phase for 5 weeks, typically 6.3 g of crude algae oil was obtained. These results compare favourably with optimized yields reported from established methods for lipid extraction.[70, 74] The composition of the oil as determined via gas chromatography after transesterification with methanol agrees with expected values[56] (**Table 3-1** and **Figure 3-2**). Gas chromatography with an internal standard showed that on average 3.3 mmol double bonds per 1 g algae oil are present.

Table 3-1: Composition of algae oil extracted from *Phaeodactylum tricornutum* (transesterified to the corresponding methyl ester for GC analysis).[a]

Fatty acid methyl ester	Content [%][b]
Myristic acid methyl ester (FA14:0)	8
Palmitoleic acid methyl ester (FA16:1)	40
Palmitic acid methyl ester (FA16:0)	32
Oleic acid methyl ester (FA18:1)	10
Eicosapentaenoic acid methyl ester (FA20:5)	9

[a]The oil was extracted via a modified Folch method. [b]Determined via gas chromatography after transesterification.

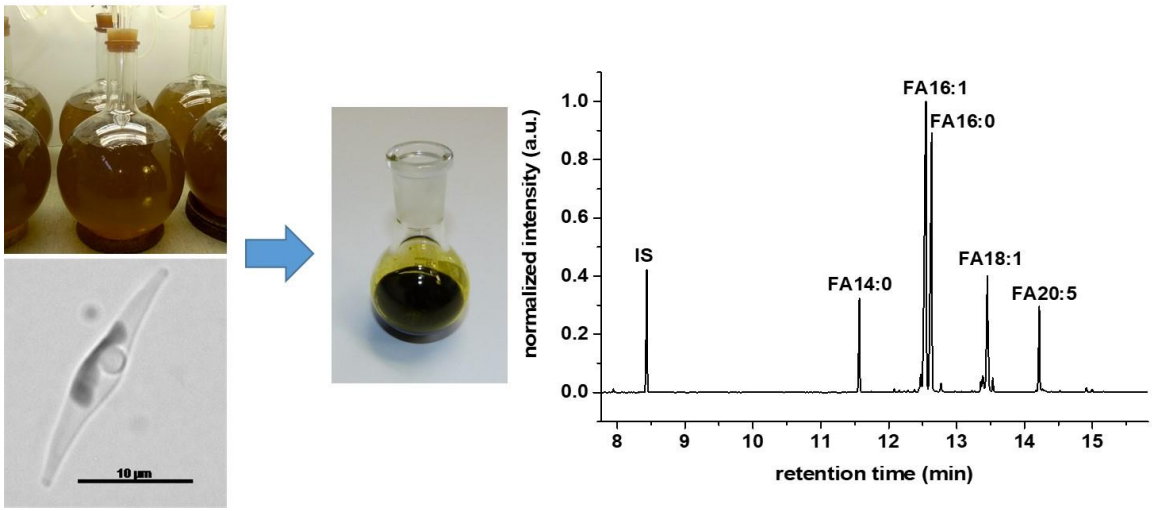


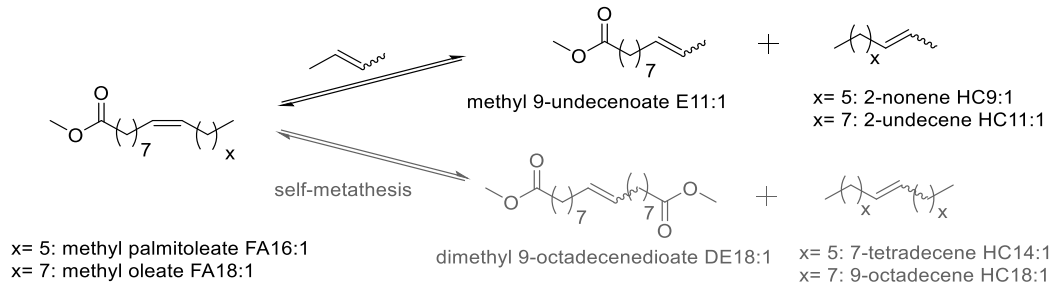
Figure 3-2: Gas chromatogram of crude algae oil extracted from *Phaeodactylum tricornutum* with nonanoic acid as internal standard (IS) (after transesterification with MeOH). Integrals of the signals are listed in Table 3-1.

3.2.2 Butenolysis of Model Compounds and Algae Oil

The fatty acids in algae oil are not only present as triacylglycerides but also as diacylglycerides, substituted with polar substituents such as galactosyl or phosphate groups on the third hydroxy moiety of glycerol.[121] Furthermore, as indicated by its green colour (c.f. **Figure 3-2**), the extracted algae oil also contains other components such as carotenoids and chlorophylls.

For identification of the possible products arising from the variety of different unsaturated fatty acids present in the crude algae oil, metathesis was conducted on selected individual fatty acid esters as model compounds.

The butenolysis of methyl oleate (FA18:1), as one mono-unsaturated fatty acid present in algae oil, was performed at -5 °C with a catalyst loading of 0.1 mol% of Hoveyda-Grubbs 2nd generation catalyst and 10 equivalents of 2-butene. Butenolysis of methyl oleate produces methyl 9-undecenoate (E11:1) and 2-undecene (HC11:1) (**Scheme 3-1**).



Scheme 3-1: Self- and cross-metathesis products of methyl oleate and methyl palmitoleate respectively with 2-butene.

By applying a tenfold excess of 2-butene, self-metathesis of methyl oleate producing dimethyl 9-octadecenedioate (DE18:1) and 9-octadecene (HC18:1) can be supressed. GC analysis (**Figure 3-3**) revealed that a conversion of 92% of the starting material was reached after already 30 minutes. The selectivity for butenolysis products (methyl 9-undecenoate (E11:1) and 2-undecene (HC11:1)) was 96%. For both products a *cis*:*trans* ratio of about 20:80 was observed. Apart from remaining FA18:1 (4%) and the desired cross-metathesis products, only small amounts of self-metathesis products were found, 1% of dimethyl 9-octadecenedioate and 1% 9-octadecene (see **Figure 3-3**).

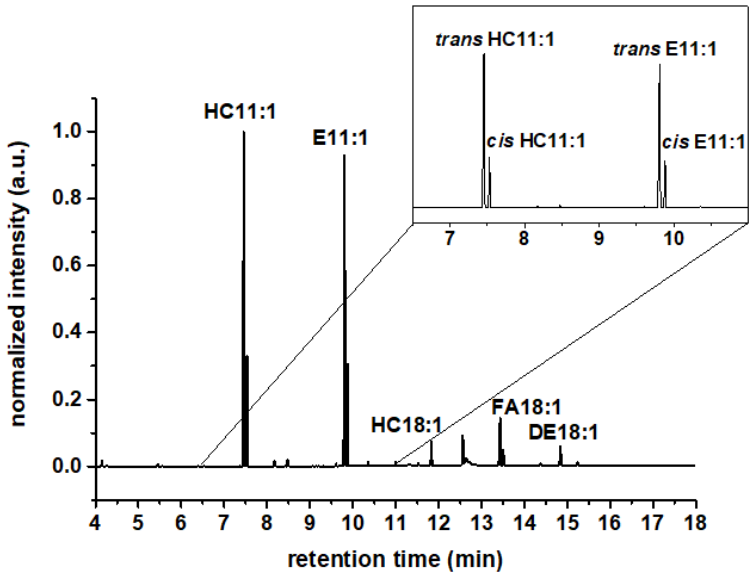


Figure 3-3: Gas chromatogram of the reaction mixture of butenolysis of methyl oleate (FA18:1) with assignment of the butenolysis products (methyl 9-undecenoate (E11:1), 2-undecene (HC11:1)) and self-metathesis products (9-octadecene (HC18:1), dimethyl 9-octadecenedioate (DE18:1)). In the insert the *trans*- and *cis*-isomers of HC11:1 and E11:1 are assigned.

Methyl palmitoleate (FA16:1, **Scheme 3-1**) was metathesized under the same conditions as for methyl oleate with a conversion of 88%. GC analysis (**Figure 3-4**) revealed a high selectivity of 88% for butenolysis products (2-nonene (HC9:1) and methyl 9-undecenoate (E11:1), **Scheme 3-1**) each with a trans *cis*:*trans* ratio of about 20:80 and only minor amounts of self-metathesis products (dimethyl 9-octadecenedioate (DE18:1) and 7-tetradecene (HC14:1)).

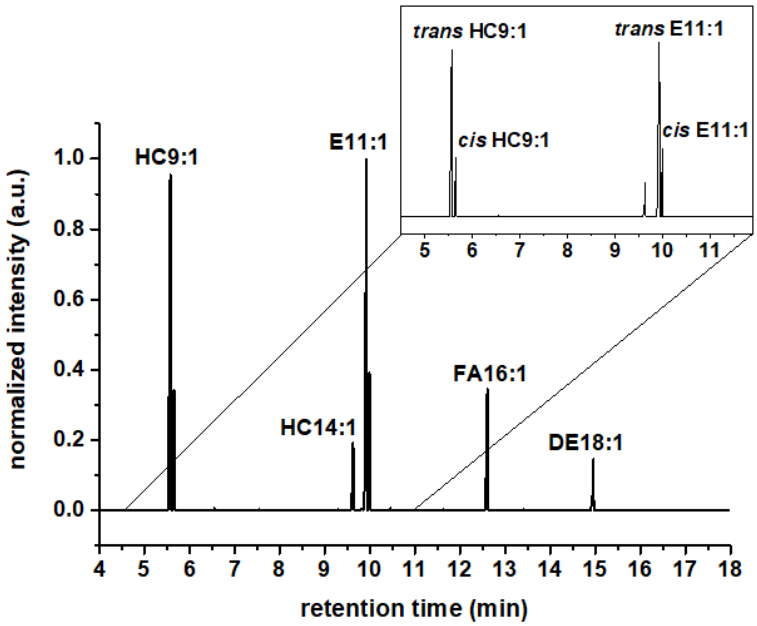
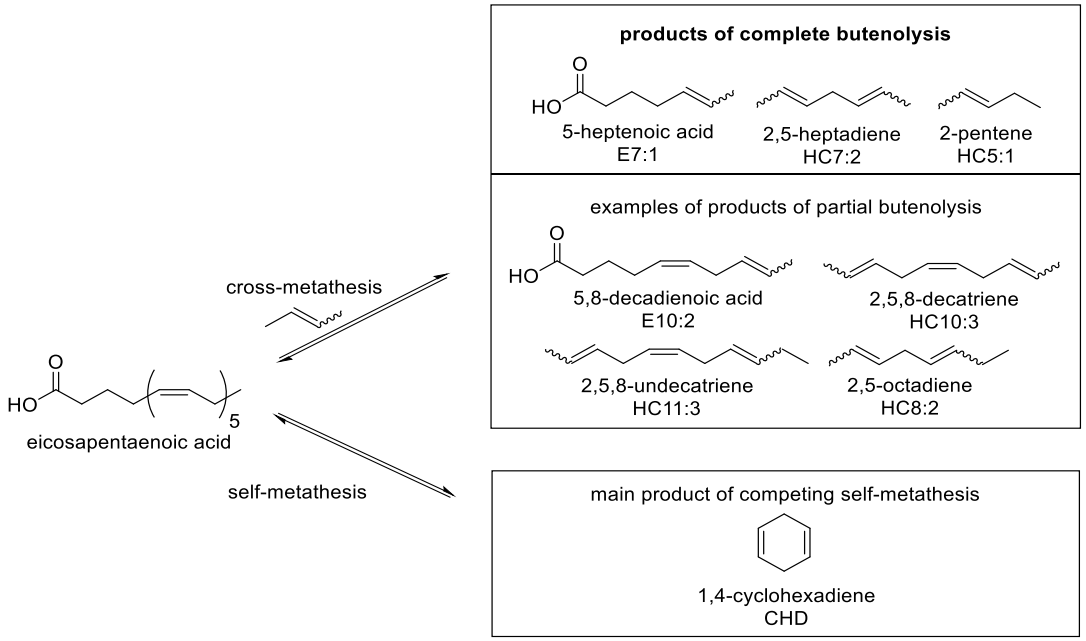


Figure 3-4: Gas chromatogram of the reaction mixture of butenolysis of methyl palmitoleate (FA16:1) with assignment of the butenolysis products (methyl 9-undecenoate (E11:1), 2-nonene (HC9:1)) and self-metathesis products (7-tetracene (HC14:1), dimethyl 9-octadecenedioate (DE18:1)). In the insert the *trans*- and *cis*-isomers of HC9:1 and E11:1 are assigned.

One unique component present in algae oil is the five-fold unsaturated fatty acid eicosapentaenoic acid (**Scheme 3-2**). Butenolysis of such poly-unsaturated fatty acids has not been reported to date, although it can give access to a range of versatile building blocks. Complete butenolysis of eicosapentaenoic acid can generate up to four equivalents of 2,5-heptadiene (HC7:2). The major challenge in cross-metathesis with eicosapentaenoic acid is the suppression of the favoured intramolecular metathesis to 1,4-cyclohexadiene (CHD).



Scheme 3-2: Cross-metathesis of eicosapentaenoic acid with 2-butene and 1,4-cyclohexadiene as main product of competing self-metathesis.

A range of ruthenium-based metathesis catalysts (**Figure 3-5**) were screened to achieve high selectivity for the desired butenolysis products.

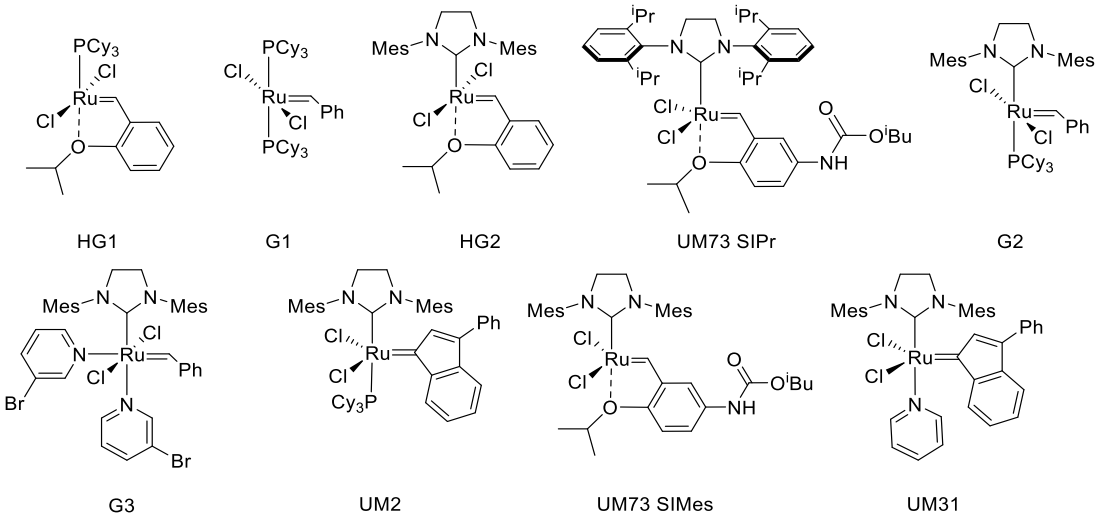


Figure 3-5: Ruthenium-based catalyst precursors. HG1: Hoveyda-Grubbs 1st gen.; G1: Grubbs 1st gen.; HG2: Hoveyda-Grubbs 2nd gen., U M73 SIPr: Umicore M73 SIPr; G2: Grubbs 2nd gen.; G3: Grubbs 3rd gen.; UM2: Umicore M2; UM73 SIMes: Umicore M73 SIMes; UM31: Umicore M31.

To compare the activity and selectivity of these different metathesis catalyst a catalyst loading of 0.1 mol% and a 10-fold excess of 2-butene with respect to the double bonds was chosen as standard conditions. All reaction mixtures were analysed via GC after transesterification with methanol (**Table 3-2**, **Figure 3-6**).

Table 3-2: Catalyst screening for butenolysis of eicosapentaenoic acid. (HG1: Hoveyda-Grubbs 1st gen.; G1: Grubbs 1st gen.; HG2: Hoveyda-Grubbs 2nd gen., UM73 SIPr: Umicore M73 SIPr; G2: Grubbs 2nd gen.; G3: Grubbs 3rd gen.; UM2: Umicore M2; UM73 SIMes: Umicore M73 SIMes; UM31: Umicore M31).

Catalyst	Conversion [%][a]	Methyl 5-heptenoate [%][b]	1,4-cyclohexadiene [%][c]
HG1	<1	-	-
G1	10	15	30
HG2	88	40	3
UM73 SIPr	52	28	1
G2	97	48	24
G3	< 1	-	-
UM2	<1	-	-
UM73 SIMes	64	28	3
UM31	22	17	1

0.1 mol% catalyst-loading per double bond, 10 equivalents 2-butene per double bond, -5 °C. [a]Reactions were stopped after no further progress was observed. [b]Product selectivity, which is defined as ratio of methyl 5-heptenoate to all formed esters. [c]Product selectivity, which is defined as ratio of 1,4-cyclohexadiene to all olefins and poly-unsaturated esters.

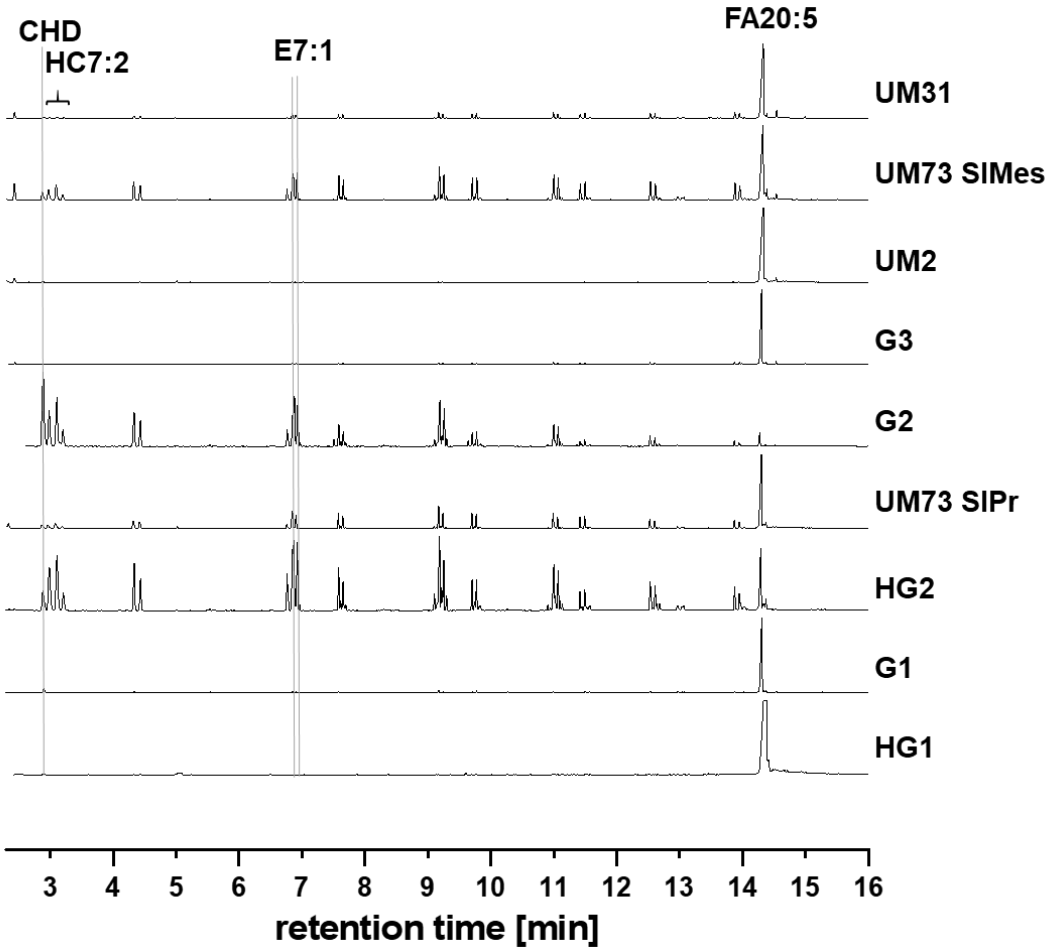


Figure 3-6: Gas chromatograms (after esterification with methanol) of catalyst screening of butenolysis of eicosapentaenoic acid with a catalyst loading of 0.1 mol% and 10 equivalents of 2-butene per double bond at -5°C. HG1: Hoveyda-Grubbs 1st Generation; G1: Grubbs 1st Generation; HG2: Hoveyda-Grubbs 2nd Generation, UM73 SIPr: Umicore M73 SIPr; G2: Grubbs 2nd Generation; G3: Grubbs 3rd Generation; UM2: Umicore M2; UM73 SIMes: Umicore M73 SIMes; UM31: Umicore M31, CHD: 1,4-cyclohexadiene, HC7:2: 2,5-heptadiene, E7:1: methyl 5-heptenoate. All non-assigned signals correspond to polyunsaturated products of incomplete butenolysis.

From this study, Hoveyda-Grubbs 2nd generation catalyst (HG2) appeared to be the most efficient in terms of conversion and selectivity for butenolysis products (**Table 3-2**). Moreover, even at high conversions only little self-metathesis occurred. Nevertheless, butenolysis was incomplete and mainly two- and three-fold unsaturated fatty acids and alkenes were obtained (c.f. **Scheme 3-2**, **Figure 3-6** HG2).

The products of butenolysis of eicosapentaenoic acid with 0.1 mol% HG2 were identified by GC-MS (see experimental section). Additionally, the reaction mixture was hydrogenated to reduce the number of compounds and for further identification (experimental section, **Figure 3-18**). This reaction mixture was also analysed by GC-MS (**Table 3-3**) and the gas chromatogram was compared to available genuine samples of individual compounds (experimental section,

Figure 3-19). Note that neither heptane nor octane, from 2,5-heptadiene and 2,5-octadiene were observed in the GC trace as the experimental procedure for hydrogenation reactions involves evacuation of the reaction vessels.

At an increased catalyst loading of 0.2 mol% per double bond, under otherwise identical conditions, eicosapentaenoic acid was completely consumed after 30 minutes and the composition of the reaction mixture did not change anymore after 120 minutes (**Figure 3-7**, top). The selectivity for methyl 5-heptenoate (E7:1), which is defined as ratio of methyl 5-heptenoate to all formed esters, increased to 95%. For methyl 5-heptenoate a *cis:trans* ratio of 17:83 was found, matching well with reported isomer ratios for similar reactions.[30] Accordingly, looking at the olefins in the final reaction mixture 2,5-heptadiene (HC7:2) was found to be the major product representing a total share of 80%. All three possible isomers of 2,5-heptadiene were observed with the *trans-trans* isomer predominating (69% *trans-trans*, 28% *trans-cis*, 3% *cis-cis*). The intramolecular formation of 1,4-cyclohexadiene (CHD) remained supressed to a large extent, only a total of 12% of all olefins found was CHD. The remainder consisted of poly-unsaturated olefins and esters from incomplete butenolysis, accounting in total for 8%. The smallest component, 2-pentene (HC5:1) resulting from the butenolysis of the chain end of eicosapentaenoic acid, was not detected in GC analysis due to its low boiling point and overlap with the solvent signal.

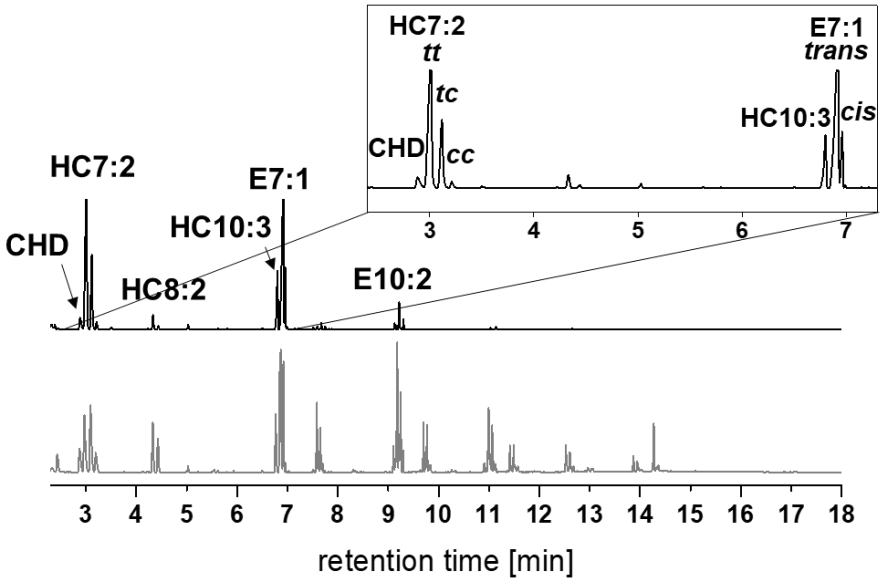


Figure 3-7: Gas chromatogram of the reaction mixture of butenolysis of eicosapentaenoic acid (after esterification with methanol) applying Hoveyda-Grubbs 2nd generation catalyst (top, black: 0.2 mol% catalyst-loading; bottom, grey: 0.1 mol% catalyst-loading) with assignment of the formed major products (1,4-cyclohexadiene (CHD), 2,5-heptadiene (HC7:2), 2,5-octadiene (HC8:2), 2,5,8-decatriene (HC10:3), methyl 5-heptenoate (E7:1), 5,8-decadienoic acid (E10:2)). In the insert the different isomers of HC7:2 (*tt*: *trans-trans*, *tc*: *trans-cis* and *cc*: *cis-cis*) and E7:1 are assigned.

In summary, butenolysis of the individual fatty acids present in algae oil proceeds with high conversion and high selectivity under the reaction conditions studied. All expected butenolysis products could be identified analytically. In addition, the favoured self-metathesis of eicosapentaenoic acid is successfully suppressed, and the poly-unsaturated nature of the fatty acid is not problematic for metathesis itself.

However, as mentioned previously, algae contain diacylglycerides with an additional polar moiety, which might interfere with catalysis. The influence of components present in algae oil, e. g. phosphocholines, were therefore investigated. Methyl oleate was used as a model substrate with additional 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (1 mol%) in a butenolysis reaction. Even in the presence of such an excess of phosphocholine (compared to the catalyst loading of 0.1 mol%) a conversion of 71% after 30 min and 90 % after 60 min was achieved. The reaction is somewhat slower compared to the pure mono-unsaturated fatty acid methyl ester, but selectivity is not compromised (94 % for butenolysis products).

With the butenolysis products of the components of algae oil identified, butenolysis was performed on the extracted crude algae oil (after degassing). A catalyst loading of 0.1 mol% and tenfold excess of 2-butene, referring to the number of double bonds, was used. This lower amount of catalyst compared to butenolysis of eicosapentaenoic acid was considered reasonable as the algae oil does not consist of eicosapentaenoic acid only but mostly of mono-unsaturated fatty acids, which could be converted with a lower catalyst loading per double bond. GC analysis revealed a virtually complete conversion of the unsaturated fatty acids (98% for the mono-unsaturated fatty acids and 100% for the five-fold unsaturated fatty acid) after 3h and showed no further change in composition of the reaction mixture. The reaction of such a complex mixture is, unsurprisingly, somewhat slower compared to the isolated fatty acids and esters. As expected a mixture of the butenolysis products of the fatty acids present in the algae oil are observed (after transesterification with methanol): 2-nonene, 2-undecene and methyl 9-undecenoate as butenolysis products of the mono-unsaturated fatty acid esters, methyl oleate and palmitoleate, and methyl 5-heptenoate and 2,5-heptadiene formed from the five-fold unsaturated fatty acid ester. In accordance to the results of the neat model compounds, all butenolysis products are obtained with the expected *cis*:*trans* ratio with the *trans* isomer predominating. Butenolysis products of the mono-unsaturated fatty acid esters were produced in 85% selectivity. For 2,5-heptadiene, a butenolysis product of FA20:5, a selectivity of 71% with respect to all olefins formed from FA20:5 was observed. Also, in this case, self-metathesis was suppressed to a large extent, only 12% of 1,4-cyclohexadiene were formed. These results are comparable to the butenolysis of pure eicosapentaenoic acid. Minor unassigned signals in the GC trace of the butenolysis product of algae oil (**Figure 3-8**) possible arise from components present in the starting algae oil, or cross-metathesis products. As expected, the content of saturated fatty acids, myristic and palmitic acid,

remained unchanged during the metathesis reaction. Overall, butenolysis of algae oil proceeds with virtually complete conversion of the unsaturated fatty acids and high selectivity to the desired ω2-unsaturated products. Hoveyda-Grubbs 2nd generation catalyst is compatible with the various components of algae oil and converts the unsaturated long chain fatty acids to a range of olefins and unsaturated acids suitable for production of higher value mid-chain chemicals.

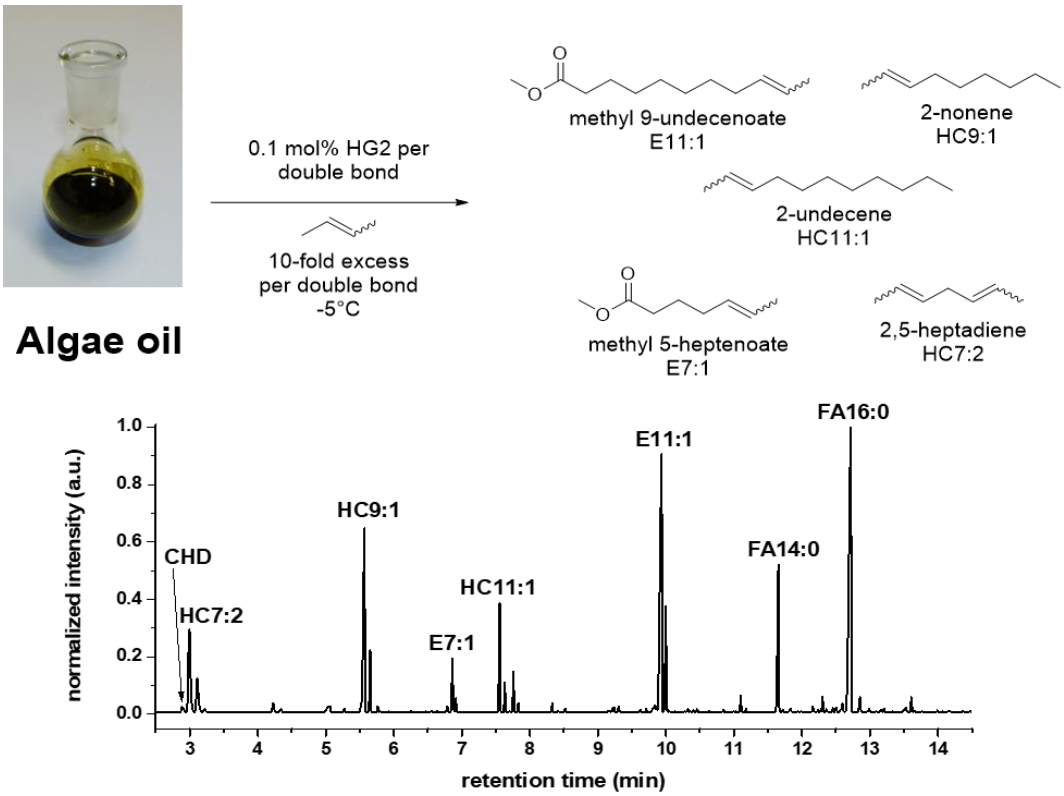


Figure 3-8: Gas chromatogram of butenolysis of crude algae oil (after transesterification with methanol) applying Hoveyda-Grubbs 2nd generation catalyst (HG2) and assignments of the butenolysis products, self-metathesis products (1,4-cyclohexadiene (CHD)) and saturated fatty acid esters (methyl myristate (FA14:0), methyl palmitate (FA16:0)).

3.2.3 Isomerizing Alkoxycarbonylation of Butenolysis Reaction Mixtures

For further upgrading of the butenolysis products, the reaction mixtures from butenolysis were subjected to isomerizing alkoxycarbonylation. With this reaction, an internal double bond is converted to a terminal ester with CO and methanol. Pd-complexes with sterically demanding diphosphine ligands, for example [Pd(dtbpx)$(OTf)_2$], catalyse this reaction with high linear selectivities. Initially, the mixture resulting from the butenolysis of the model compound eicosapentaenoic acid was subjected to isomerising alkoxycarbonylation under conditions previously reported for seed oils (90 °C, 20 bar CO, 0.8 mol% [Pd(dtbpx)$(OTf)_2$]).[122,123] Mainly 2,5-heptadiene and 2-heptenoic acid were present in the starting mixtures, which will produce

dimethyl nonanedioate (DE9:0, **Figure 3-9**) and dimethyl octanedioate (DE8:0, **Figure 3-9**), respectively. Additionally, methyl hexanoate (E6:0, **Figure 3-9**) as an alkoxycarbonylation product from 2-pentene can be expected. For all alkoxycarbonylation reactions, the selectivity to a product is defined as the ratio of the product to the theoretical maximum amount of this product based on the product mixture obtained in the metathesis step and is determined over an internal standard. 2-Heptenoic acid was completely converted after 3 days to the linear DE8:0 in 90% selectivity. Methyl hexanoate (E6:0), as the alkoxycarbonylation product of 2-pentene, was formed with a selectivity of 58% in respect to the amount of eicosapentaenoic acid in the starting material employed for butenolysis. This is a reasonable amount, considering the volatility and low boiling point of 2-pentene which can result in losses of this intermediate in the protocol applied. The conversion of heptadiene was somewhat slower, it was fully converted after 7 days to DE9:0 in 58% overall selectivity. In addition, methyl pentanoate was found, which results from the isomerizing alkoxycarbonylation of remaining 2-butene.

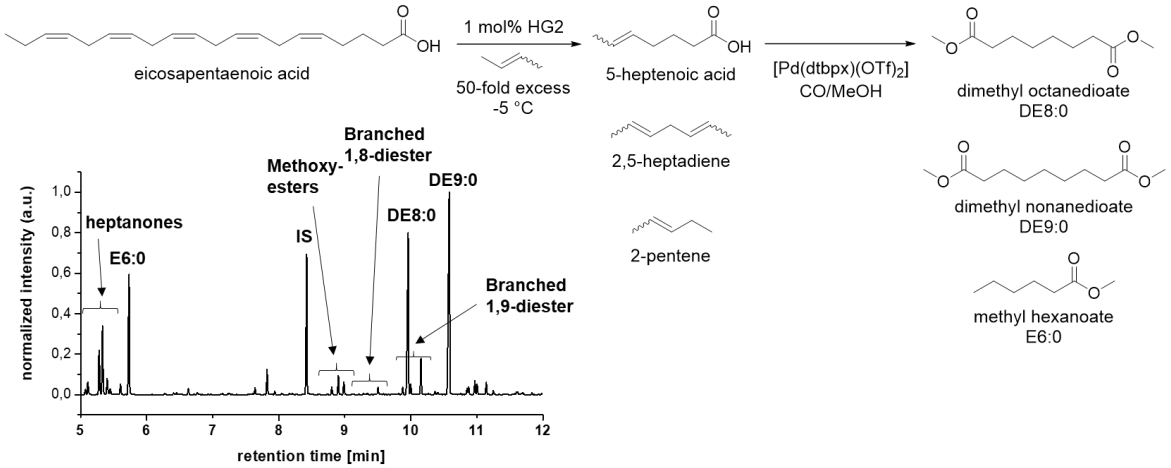


Figure 3-9: Gas chromatogram of methoxycarbonylated butenolysis products of eicosapentaenoic acid with nonanoic acid as an internal standard (IS) and Hoveyda-Grubbs 2nd generation catalyst (HG2). Structures of the butenolysis products of eicosapentaenoic acid and the corresponding alkoxycarbonylation products.

The limited selectivity for DE9:0 can be traced to the two double bonds in heptadiene, which can form a Pd-allyl complex. Such complexes are known to decrease the rate of the alkoxy-carbonylation significantly.[124] Because of the lower reaction rate, side reactions can become relevant to some extent affecting the selectivity towards the linear 1,9-diester.

To investigate this lower selectivity, 2,5-heptadiene was isolated from the butenolysis reaction mixture and the isomerizing alkoxycarbonylation of this compound was investigated in detail.

Heptadiene was isolated from the butenolysis reaction mixture by column chromatography with pentane as an eluent. A part of the 2,5-heptadiene (21%) isomerized to the conjugated 2,4-heptadiene (see **Figure 3-10** and **Figure 3-11**) and due to the low vapour pressure of heptadiene the eluent pentane could not be removed completely.

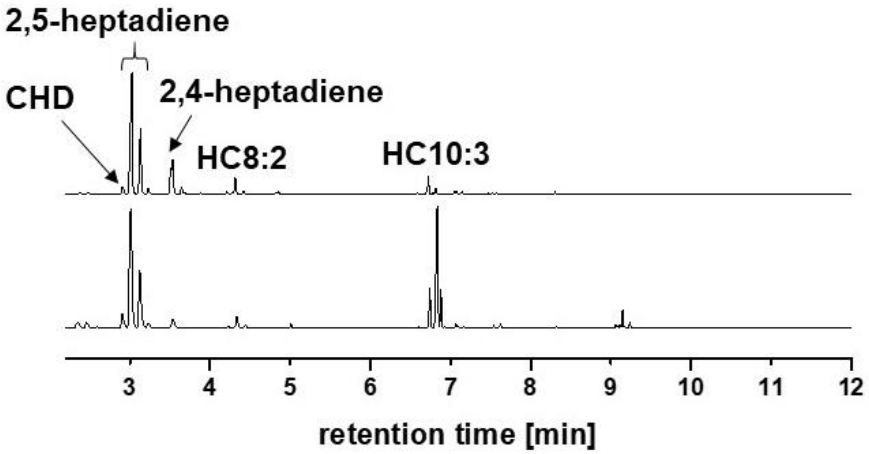


Figure 3-10: Gas chromatogram of the butenolysis reaction mixture of eicosapentaenoic acid after esterification with methanol (bottom) and of the heptadiene separated by column chromatography (top).

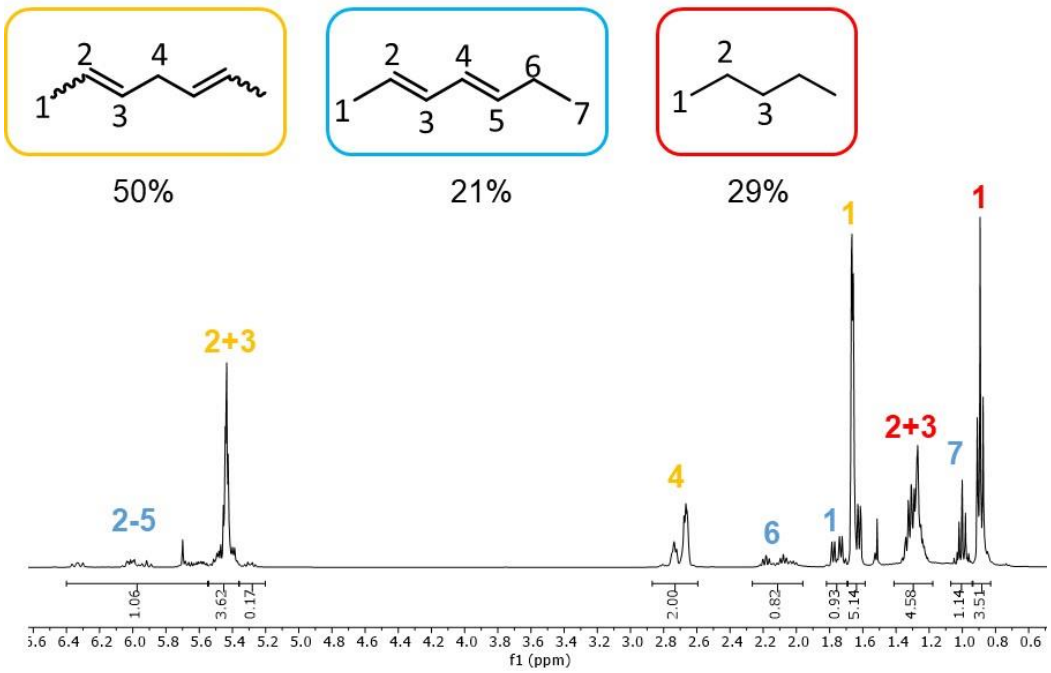


Figure 3-11: ^{1}H-NMR ($CDCl_3$, 400 MHz, 302 K) spectrum of the isolated heptadiene fraction.

Alternatively, heptadiene can also be separated by distillation under reduced pressure (**Figure 3-12**) as well as distillation at ambient pressure (**Figure 3-13**). At ambient pressure and

therefore higher temperature (64-78 °C) isomerization is more pronounced (61% vs. 6%). Yet, in general isomerization to 2,4-heptadiene is not disadvantageous to the outcome of the alkoxycarbonylation, as under alkoxycarbonylation conditions a rapid isomerization usually occurs in any case (**Figure 3-14**).

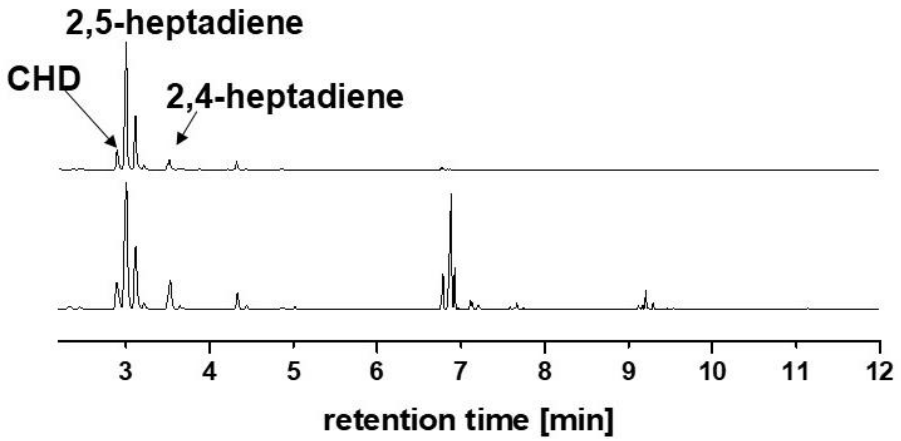


Figure 3-12: Gas chromatogram of the butenolysis reaction mixture of eicosapentaenoic acid after esterification with methanol (bottom) and of the distillate obtained by vacuum distillation (top).

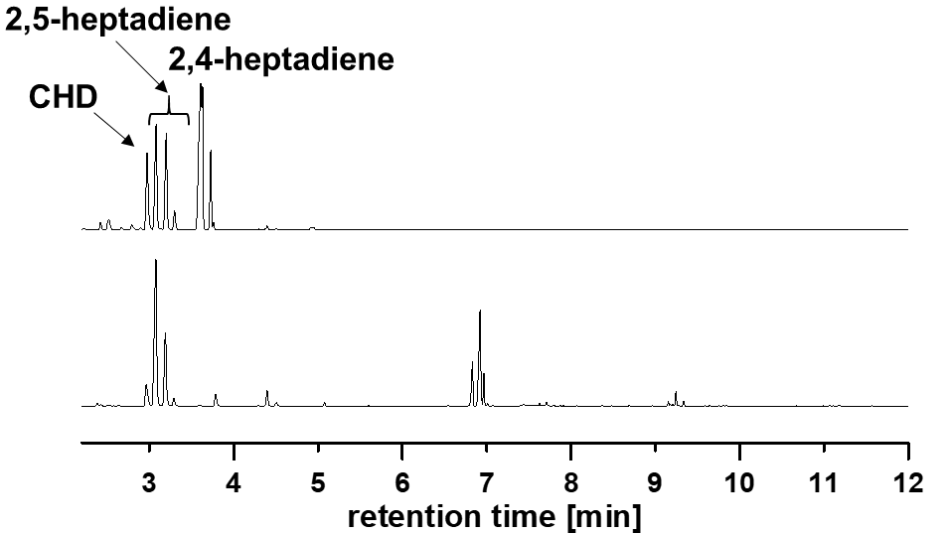


Figure 3-13: Gas chromatogram of the butenolysis reaction mixture of eicosapentaenoic acid after esterification with methanol (bottom) and gas chromatogram of the distillate obtained by distillation at ambient pressure and 64-78 °C boiling range (top).

The isolated mixture of heptadienes (2,5- and 2,4-heptadiene) was subjected to isomerizing alkoxycarbonylation under the standard conditions and monitored over time (**Figure 3-14** and **Figure 3-15**). The samples were analysed by GC, GC-MS and NMR. Besides the expected linear 1,9-diester (DE9:0, **Figure 3-16**) as main product, which is formed with a selectivity of 55%, also branched diesters (**Figure 3-16**) were observed in 10% selectivity which is typical for this

catalyst for mono-unsaturated substrates.[125] For the largest part (7%) these are composed of dimethyl 2-methyloctandioate. Further, three different ketones (2-heptanone, 3-heptanone and 4-heptanone, **Figure 3-16**) were identified by enrichment experiments with genuine samples (see chapter 3.4.6, **Figure 3-22**). These ketones made up 24% of the formed products and are formed via addition of residual water to a double bond, followed by isomerization to the enol. Via tautomerization this subsequently forms the saturated ketone.[126] Finally, based on GC-MS data (see experimental section) it can be assumed that the remainder are methyl methoxyoctanoates (methoxy-esters, 11%, **Figure 3-14** and **Figure 3-16**), from nucleophilic addition of methanol and only one isomerizing alkoxycarbonylation step of heptadiene.

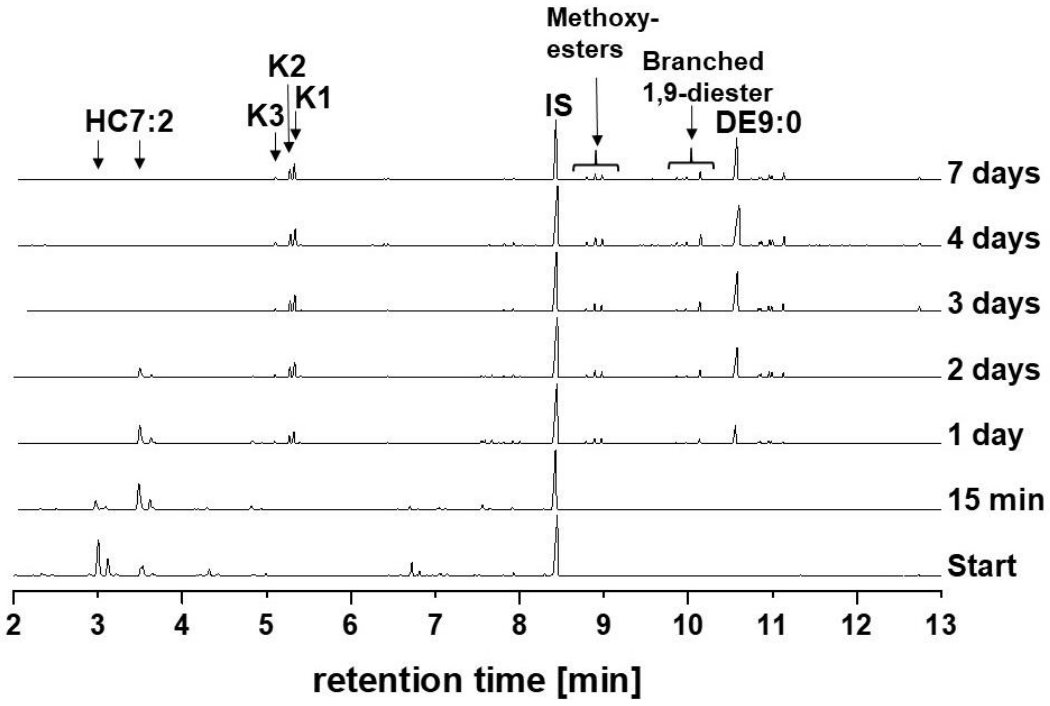


Figure 3-14: Gas chromatograms of isomerizing alkoxycarbonylation of a mixture of 2,4- and 2,5-heptadiene (HC7:2) (0.8 mol% [Pd(dtbpx)(OTf)$_2$], 20 bar CO, 90 °C, nonanoic acid as an internal standard (IS)).

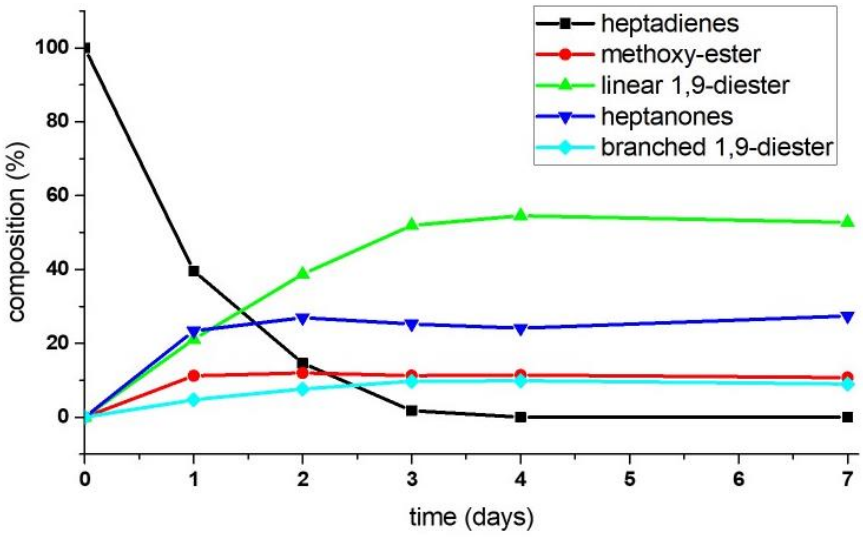


Figure 3-15: Reaction profile of the alkoxycarbonylation of heptadiene. Conditions: 0.8 mol% [Pd(dtbpx)(OTf)$_2$], Substrate, MeOH, 20 bar CO, 90 °C. ■ = heptadiene, ▲ = 1,9-diester, ▼ = ketones, ● = methoxy esters, ♦ = branched diesters.

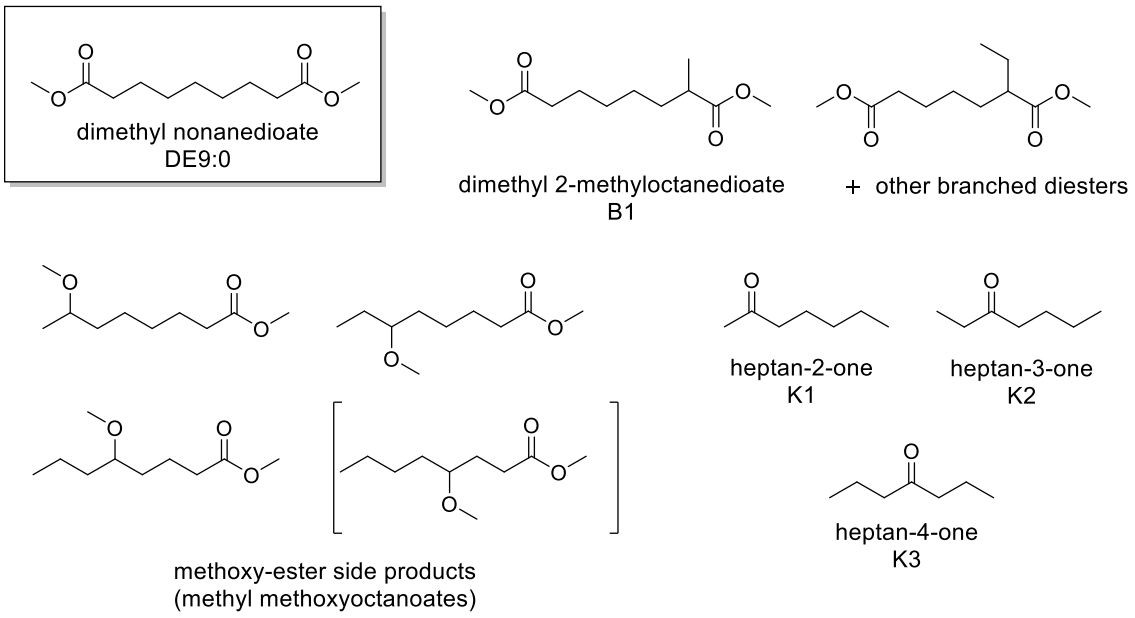


Figure 3-16: Structures of products of isomerizing alkoxycarbonylation of a mixture of 2,4- and 2,5-heptadiene.

With all products identified from these experiments, the reaction mixture of the butenolysis of algae oil was subjected to the isomerizing alkoxycarbonylation without any intermediate purification step. All butenolysis products were converted after three days. The butenolysis products from the mono-unsaturated fatty acid esters, methyl oleate and methyl palmitoleate, are 2-undecene and 2-nonene, respectively, and for both methyl undecenoate. These butenolysis

products are expected to be converted into methyl dodecanoate (E12:0), methyl decanoate (E10:0) and dimethyl dodecanedioate (DE12:0) (**Figure 3-17**). The selectivity was determined based on the theoretical maximum value in case of complete conversion of the corresponding butenolysis products. The selectivity for methyl dodecanoate (DE12:0), the alkoxycarbonylation product of 2-undecene formed from FA18:1, is 92%. Methyl decanoate, which is formed from 2-nonene (butenolysis product of FA16:1), was found in a selectivity of 86%. These selectivities are in accordance with literature values for the isomerizing alkoxycarbonylation of a neat linear mono-unsaturated substrate.[125] DE9:0 the alkoxycarbonylation product of HC7:2 is formed with a selectivity of 61%. This is in accordance to the selectivity observed for the isomerizing alkoxycarbonylation of isolated heptadiene as previously described in this chapter. The isomerizing alkoxycarbonylation catalyst was compatible with remaining components in algae oil and was also not disturbed by the quenched metathesis catalyst.

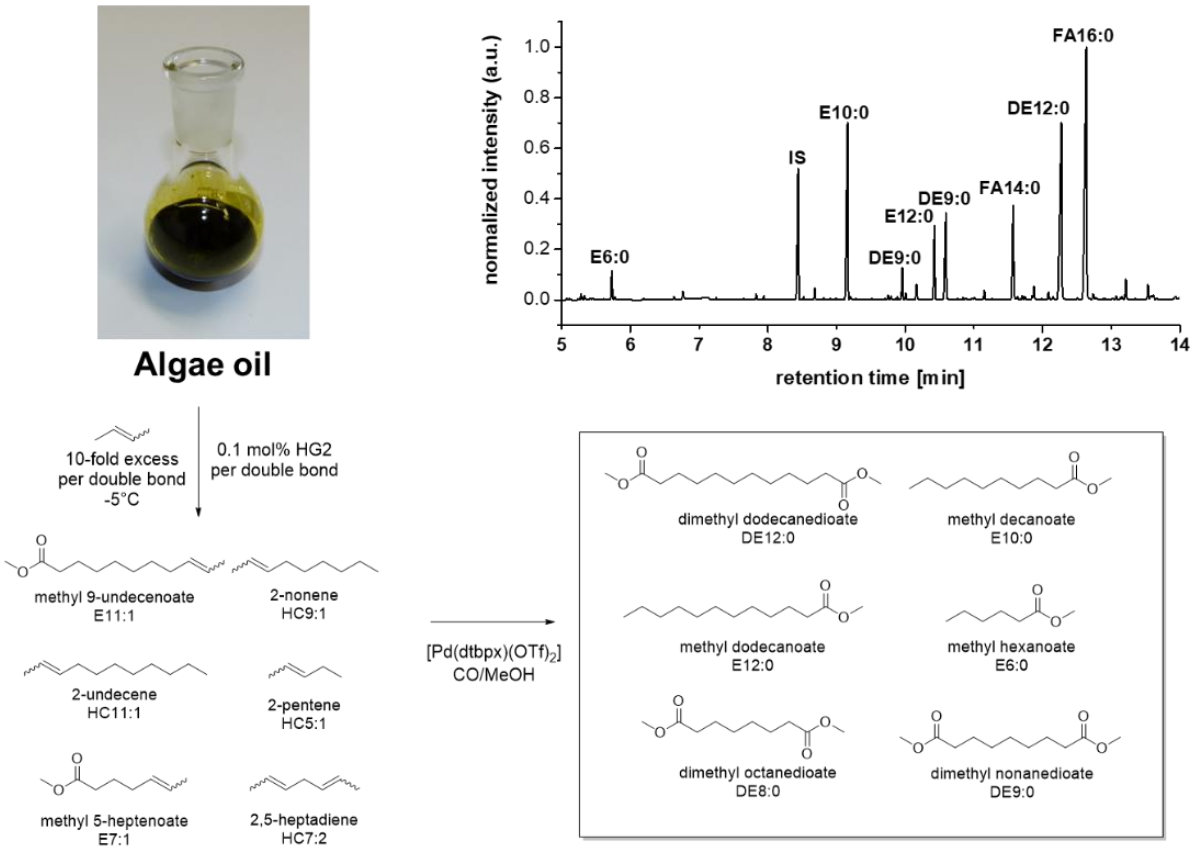


Figure 3-17 Gas chromatogram of methoxycarbonylated butenolysis products of crude algae oil and the assigned products.

3.3 Conclusion

Microalgae oil from *Phaeodactylum tricornutum* can serve as a feedstock for a biorefinery approach to mid-chain (di)carboxylic acid esters, currently only accessible via demanding synthetic routes. Via the butenolysis of mono- and poly-unsaturated fatty acid esters, short-chain unsaturated fatty acid methyl esters as well as mono- and dienes were produced in a high selectivity. Hoveyda-Grubbs 2nd generation metathesis catalyst appeared to be compatible with the multi-component mixture consisting of numerous lipids and non-lipid compounds and leads to quantitative conversions and high selectivities. Notably, also the highly unsaturated eicosapentaenoic acid is converted selectively, with 2,5-heptadiene as the major product. The olefins were further processed into value added linear mid-chain (di-)carboxylic acid esters via isomerizing alkoxycarbonylation. In the isomerizing alkoxycarbonylation of the crude butenolysis mixture, the catalyst was also compatible with "non-lipid" components in algae oil. The consecutive butenolysis and alkoxycarbonylation can be performed as a one-pot reaction, without intermediate purification steps of the crude starting material. This approach opens new possibilities to carboxylic mono- and diacid esters of chain length ranging from C8 to C12, fully based on algae oil. The resulting mono-carboxylic acid esters can serve as surfactants (E12:0 and E10:0) or food additives (E6:0), while dicarboxylic acid esters are useful as lubricants or monomers in polycondensation reactions. The C9 diester azelaic acid ester (DE9:0) is interesting especially, as current production requires the technically challenging and potentially hazardous ozonolysis. Moreover, our route provides an additional approach to other diesters (DE10:0 and DE12:0) and also a new, potentially interesting diester, DE8:0. The entire two-step catalytic route yields a number of desirable products (**Figure 3-17**) by exploiting the entire fatty acid spectrum present in algae oil from *Phaeodactylum tricornutum*.

3.4 Experimental Section

3.4.1 General Methods and Materials

All catalyst precursors used were stored in a glovebox under a nitrogen atmosphere. All reactions were conducted under inert gas atmosphere (argon or nitrogen) using standard glovebox or Schlenk techniques. Dichloromethane was dried over CaH_2 and distilled under a nitrogen atmosphere. Methanol was dried over magnesium and distilled under a nitrogen atmosphere. All other solvents were standard analytical grade and used as received. CO (3.7) was purchased from Air Liquide, *cis*/*trans*-2-butene (99 %) and n-tetradecane from ABCR GmbH. Nonanedioic acid, hexanoic acid, octanedioic acid and nonanoic acid were purchased from Fluka. Technical grade high oleic sunflower oil methyl ester (DAKOLub MB9001) kindly donated by DAKO AG was used rather than highly purified methyl oleate. The typical composition of Dakolub MB9001 is 92.5% methyl oleate, 2.5% methyl linoleate, 2.5% methyl palmitate, 1.5% methyl stearate and 1.0% longer fatty acid ester. The compound was distilled under a nitrogen atmosphere prior to use. Methyl palmitate, methyl palmitoleate and methyl myristate were purchased from Nu-Check Prep, Inc. Methyl palmitoleate was purified by Kugelrohr distillation. Eicosapentaenoic acid purchased from Carbosynth was distilled prior to use. Hoveyda-Grubbs 2nd generation was purchased from Carbosynth. 2-, 3- and 4-heptanone, 1,4-cyclohexadiene, decanoic acid, methyl laurate, decane, hexadecane, heptadecane and the metathesis catalysts, Grubbs 1st generation, Grubbs 2nd generation, Grubbs 3rd generation and Hoveyda-Grubbs 1st generation were purchased from Sigma-Aldrich. Catalyst precursors Umicore M73 SIPr, Umicore M2, Umicore M73 SIMes and Umicore M31 were kindly donated by Umicore. 1,3-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was kindly donated by Lipoid GmbH and Corden Pharma Switzerland LLC. Gas chromatography was performed on a Perkin-Elmer GC Clarus 500 equipped with an elite-5 column (Length = 30 m, Inner Diameter = 0.25 mm, Thickness = 25 μ) using helium as the carrier gas at a flow rate of 1.5 mL min^{-1}and a FID-detector via the following program: 3 min isothermal at 50 °C, 20 °C min^{-1} to 280 °C, 280 °C for 5 min with an injector temperature of 300 °C and a detector temperature of 280 °C. All free acids were esterified with methanol prior to GC analysis. GC-MS measurements were conducted on an Agilent GC7890A system equipped with an inert MSD 5975C triple-axis detector. GC-MS was performed with a HP-5ms column. NMR spectra were recorded on a Bruker Avance III 400 MHz. ^{1}H NMR spectra were referenced to residual protonated solvent ($CDCl_3$).

3.4.2 Algae Cultivation and Extraction

Phaeodactylum tricornutum was cultivated in 10 L flasks continuously in modified f2 cultivation medium[127] aerated with sterile ambient air in a day/night rhythm of 16/8 hours at 20 °C and with a light intensity of 35 µmol x s^{-1} x m^{-2}.

30 L of algae culture were centrifuged for 10 min at 4000 g and 4 °C using a Sorvall RC 6 Plus centrifuge equipped with a Sorvall SLA 3000 rotor. The obtained cell pellet was stored at -28 °C. The oil was extracted via a modified method of Folch *et al*.[69] The cell pellet (48.6 g) was thawed and diluted with 30 mL ddH_2O. The mixture was put on ice and pre-treated by ultrasonication for 10 min with a pulse of 10 s and amplitude of 60%, using an ultrasound homogenizer D2200 from BANDELIN with a KE76 sonotrode. 190 mL Chloroform and 90 mL methanol were then added to establish a ratio of $CHCl_3$:MeOH:ddH_2O = 8:4:3. The natural water content is supposed to be approximately 75%. The organic phase containing the lipids was collected and dried over $MgSO_4$. After removal of the solvent under reduced pressure, 6.3 g algae oil was obtained.

3.4.3 General Procedure for Butenolysis

A Schlenk flask was sealed with a septum, evacuated and cooled in a cold bath (isopropanol/nitrogen) and 2-butene was condensed into the cooled Schlenk flask. Via syringe, the fatty acid (ester) or algae oil (with additional dichloromethane) and nonanoic acid as internal standard were added (ratio 2-butene per double bond = 10:1). In the experiment testing the effect of phosphocholine, 1 mol% of 1,3-dipalmitoyl-sn-glycero-3-phosphocholine was added. The flask was transferred to an ice/salt bath (-5 °C). In a separate Schlenk tube, the appropriate amount of Hoveyda-Grubbs 2nd generation catalyst was dissolved in dichloromethane and added via syringe to the pre-cooled Schlenk flask containing the butene. A catalyst loading of 0.1 mol% for the algae oil, methyl oleate and palmitoleate and for eicosapentaenoic acid 0.1 mol% or 0.2 mol% per double bond was used. Samples were drawn with a pre-cooled, airtight syringe. The samples were quenched with ethyl vinyl ether and analysed by GC. For GC analysis the samples were trans-esterified first by treating the sample with methanol/dichloromethane and a catalytic amount of sulphuric acid followed by heating. The excess of 2-butene was distilled at ambient temperature under a slight vacuum and was reused without further purification.

3.4.4 General Procedure for Isomerizing Methoxycarbonylation

Isomerizing alkoxycarbonylation reactions 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. The catalyst precursor was weighed into a Schlenk tube, and MeOH as a solvent and the reaction mixture from butenolysis were added. This mixture was transferred via syringe into the reactor. The reaction mixture was stirred at 90 °C and 20 bar CO for 7 days. The reaction mixture was diluted with CH_2Cl_2 and filtered to remove solids. Samples were taken directly from the reaction mixture and analysed by GC.

3.4.5 Identification of Butenolysis Products

To identify all butenolysis products, the retention times of the expected products were compared to genuine samples or enrichment experiments were conducted. The reaction mixture of butenolysis of eicosapentaenoic acid was hydrogenated prior to this. Additionally, GC-MS was measured.

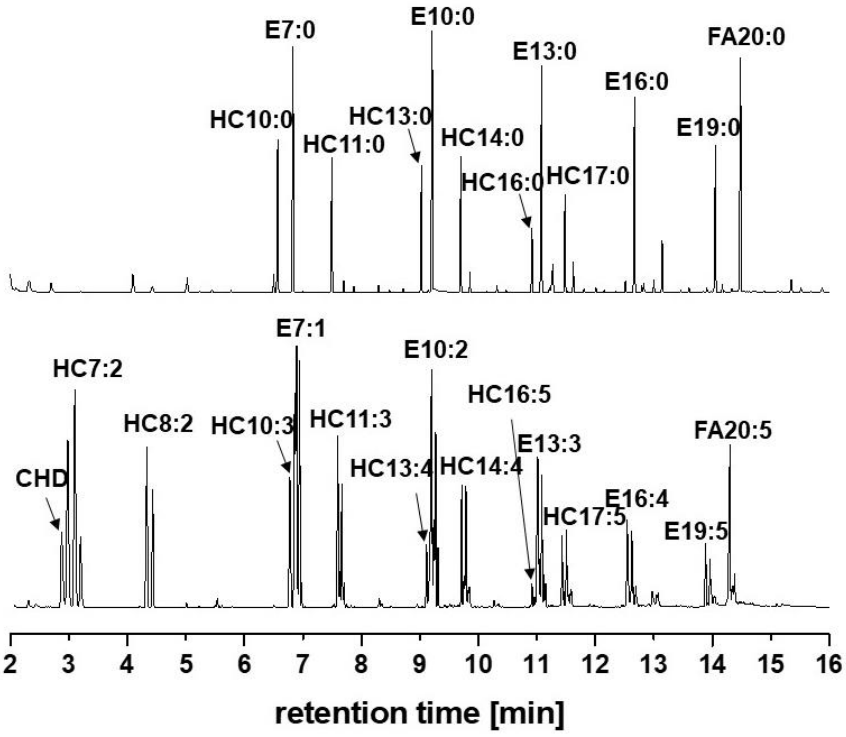


Figure 3-18: Gas chromatogram of reaction mixture of butenolysis of eicosapentaenoic acid (after esterification with methanol) applying 0.1 mol% Hoveyda-Grubbs 2nd generation catalyst (top) and the gas chromatogram after hydrogenation (bottom).

Table 3-3: Identification of butenolysis products of eicosapentaenoic acid and the corresponding products after subsequent hydrogenation and esterification by GC-MS.

Compound	Abbreviation	Calculated molecular weight [g mol^{-1}]	Mass found
2,5-Heptadiene	HC7:2	96.17	96.1
2,5-Octadiene	HC8:2	110.20	110.1
2,5,8-Decatriene	HC10:3	136.24	136.1
Methyl 5-heptenoate	E7:1	142.20	142.1
2,5,8-Undecatriene	HC11:3	150.27	150.0
2,5,8,11-Tridecatetraene	HC13:4	176.30	176.1
Methyl 5,8-decadienoate	E10:2	182.26	182.1
2,5,8,11-Tetradecatetraene	HC14:4	190.33	157.1 (M-CH_3), 161.1 (M-CH_2CH_3), 147.1 (M-$CH_2CH_2CH_3$)
2,5,8,11,14-Hexadecapentaene	HC16:5	216.37	201.0 (M-CH_3), 187.0 (M-CH_2CH_3), 174.0(M-$CH_2CH_2CH_3$)
Methyl 5,8,11-tridecatrienoate	E13:3	222.33	222.1
2,5,8,11,14-Heptadecapentaene	HC17:5	230.4	215.1 (M-CH_3), 201.1 (M-CH_2CH_3), 187.0 (M-$CH_2CH_2CH_3$)
Methyl 5,8,11,14-hexadecatetraenoate	E16:4	262.39	262.1
Methyl 5,8,11,14,17-nonadecapentaenoate	E19:5	302.46	273.1 (M- CH_2CH_3), 247.0 (M-$CH_2CHCHCH_3$)
Decane	HC10:0	142.29	142.1
Methyl heptanoate	E7:0	144.21	144.0
Undecane	HC11:0	156.31	156.1
Tridecane	HC13:0	184.37	184.2
Methyl decanoate	E10:0	186.3	186.1
Tetradecane	HC14:0	198.39	198.1
Hexadecane	Hc16:0	226.45	226.1
Methyl tridecanoate	E13:0	228.38	228.2
Heptadecane	HC17:0	240.48	240.2
Methyl hexadecanoate	E16:0	270.46	270.1
Methyl heptadecanoate	E17:0	284.48	284.1
Methyl nonadecanoate	E19:0	312.54	312.2

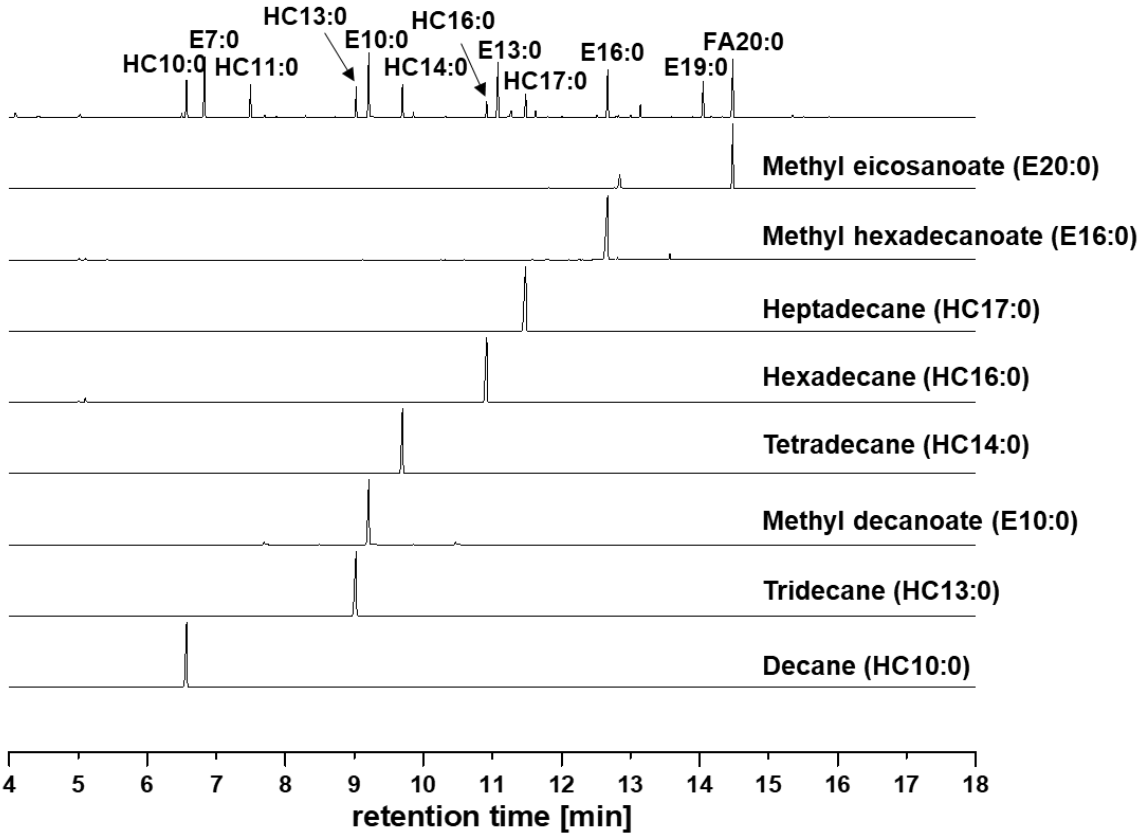


Figure 3-19: Gas chromatogram of the hydrogenated reaction mixture of butenolysis of eicosapentaenoic acid (after esterification with methanol) with 0.1 mol% Hoveyda-Grubbs 2nd generation catalyst (top) and gas chromatograms of genuine samples for identification of the hydrogenated products.

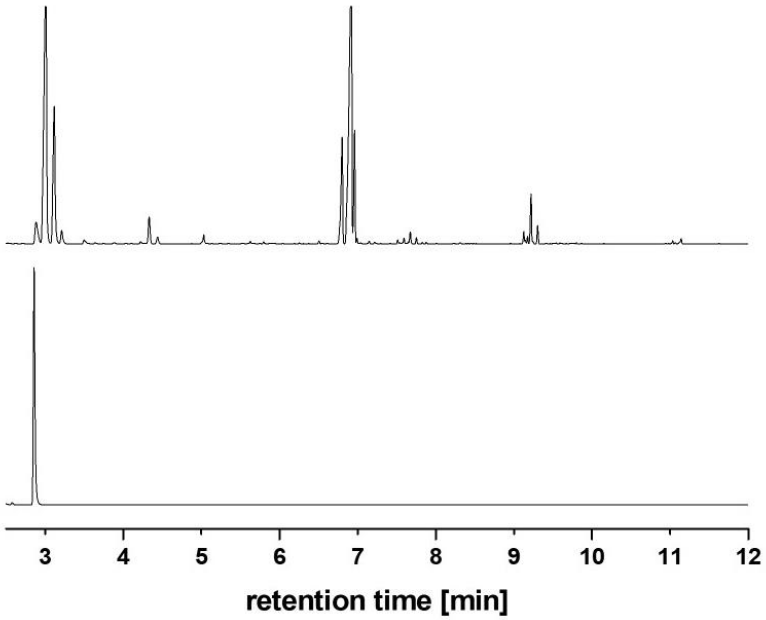


Figure 3-20: Gas chromatogram of butenolysis of eicosapentaenoic acid after esterification with methanol (top) and of a genuine sample of 1,4-cyclohexadiene (bottom).

3.4.6 Identification of Isomerizing Alkoxycarbonylation Products

To identify all carbonylation products, the retention times of the expected products were compared to genuine samples or enrichment experiments were conducted. Additionally, GC-MS was measured.

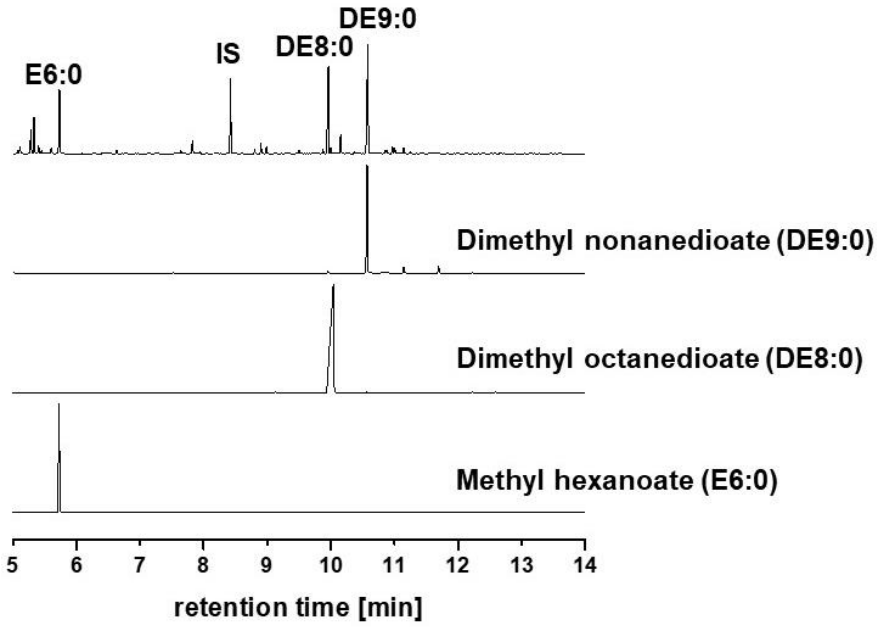


Figure 3-21: Gas chromatogram of methoxycarbonylated butenolysis products of eicosapentaenoic acid (top) with nonanoic acid as an internal standard (IS) and gas chromatograms of genuine samples of methyl hexanoate (E6:0), dimethyl octanedioate (DE8:0) and dimethyl nonanedioate (DE9:0).

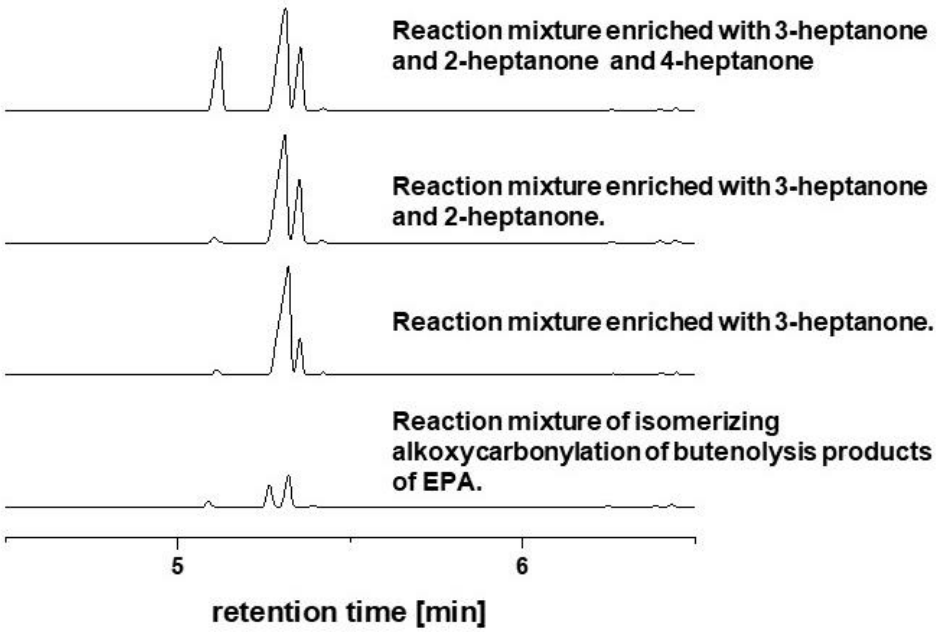


Figure 3-22: Gas chromatograms of methoxycarbonylated butenolysis products of eicosapentaenoic acid (bottom) and enrichment experiments with 2-, 3- and 4-heptanone.

The methoxy-ester side products (**Figure 3-17**) were identified by GC-MS:

Methyl methoxyoctanoate M= 188.27 g mol^{-1}; found 187.1 (M-H), 173.1 (M-CH_3), 159.0 (M-CH_2CH_3), 145.1 (M-$CH_2CH_2CH_3$), 127.1 (M-2x OCH_3), 87.1 ($\bullet CH_2CH_2COOCH_3$)

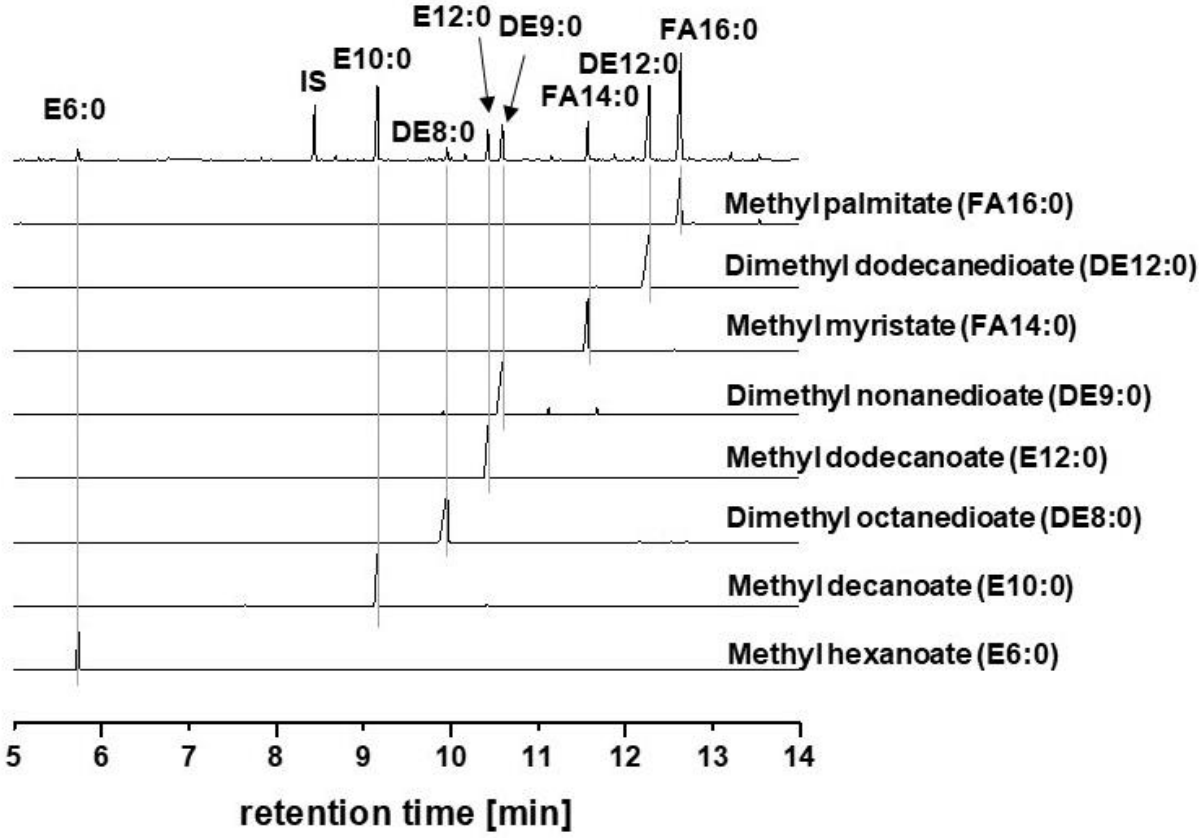


Figure 3-23: Gas chromatogram of methoxycarbonylated butenolysis products of crude algae oil with nonanoic acid as an internal standard (IS) and gas chromatograms of genuine samples of methyl hexanoate (E6:0), methyl decanoate (E10:0), dimethyl octanedioate (DE8:0), methyl dodecanoate (E12:0), dimethyl nonanedioate (DE9:0), methyl myristate (FA14:0), dimethyl dodecanedioate (DE12:0) and methyl palmitate (FA16:0).

4 Short- and Mid-Chain Olefins and Carboxylic Acid Esters by Ethenolysis and Isomerization

4.1 Introduction

Ethenolysis is a very useful transformation to upgrade fatty acids that yields terminal 1-olefinic products. Several examples of ethenolysis of fatty acids from plant oils are known in literature.[18, 22, 27] In contrast, ethenolysis of microalgae oil is less common in literature. One example is the ethenolysis of microalgae oil for the production of biofuels reported by Chuck *et al.*[78] For the cross-metathesis of microalgae oil from *Pseudochorisystis ellipsoidea* with ethylene a selectivity of 35-40% for terminal double bonds was reported.

The product scope accessible by ethenolysis of fatty acids depends on the starting material and the chain length of the formed products depend on the position of the double bond in the starting material. Based on fatty acids naturally occurring in plants typically products of even number carbon atoms are obtained. However, a broader spectrum of products can be achieved with an additional double bond isomerization step. For the combination of double bond isomerization and metathesis some examples are known in literature (see chapter 1.1.3). Gooßen *et al.* demonstrated this concept for the conversion of fatty acids to obtain defined distributions of unsaturated compounds that can be useful as biodiesel (**Figure 4-1**). The isomerizing self-metathesis of oleic acid with a NHC-indenylidene ruthenium metathesis catalyst and a dimeric palladium(I) complex $[Pd(\mu\text{-}Br)P^tBu_3]_2$ as additional isomerization catalyst was reported.[40] In this way, oleic acid was converted into a defined distribution of unsaturated compounds (C8-C32 olefins, C13-C25 monocarboxylates and C13-C22 α,ω-dicarboxylates). Additionally, the isomerizing cross-metathesis of oleic acid and different olefins such as ethylene or *trans*-3-hexenedioic acid were demonstrated. Thereby the chain lengths were significantly shorter compared to self-metathesis and moreover adjustable by the ratio of the starting materials.

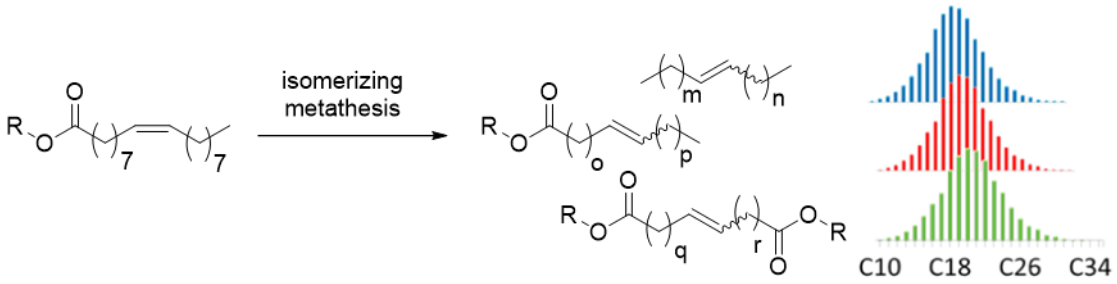


Figure 4-1: Isomerizing self-metathesis of oleic acid derivatives, together with expected product distributions for an ideal catalytic system. Reprinted with permission from reference 40. Copyright 2012 American Chemical Society.

Palladium(II) catalysts modified with electron-rich, very bulky substituted diphosphine ligands are known to be highly active in the isomerization of double bonds.[128] A prominent example which is also used in the isomerizing alkoxycarbonylation is [Pd(dtbpx)(OTf)$_2$]. Furthermore, this complex is known to be compatible with microalgae oil containing, besides fatty acids, various compounds such as pigments.[68]

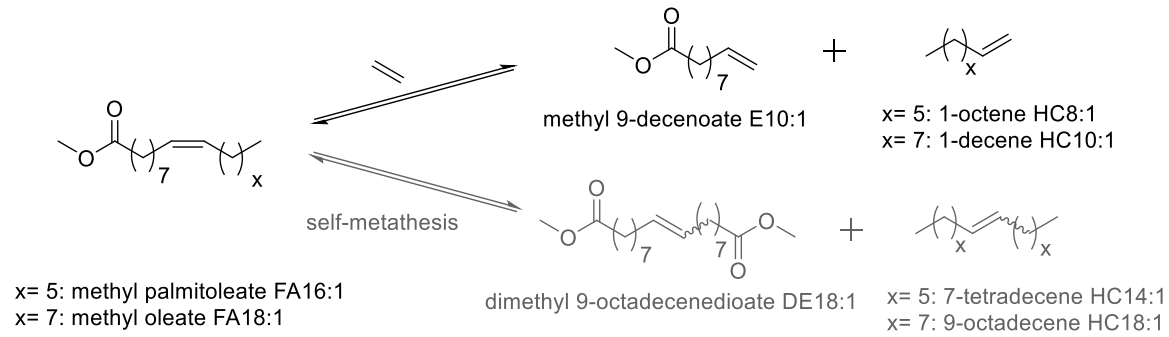
In this chapter the conversion of microalgae oil in an ethenolysis reaction towards terminal unsaturated esters and olefins is discussed. Furthermore, the concept of isomerizing ethenolysis was demonstrated on the model substrate methyl oleate leading to an extended spectrum of products with different chain length otherwise only accessible via cracking.

4.2 Results and Discussion

4.2.1 Ethenolysis of Model Compounds and Algae Oil

Catalytic upgrading of fatty acids from algae oil via ethenolysis gives access to a broad spectrum of unsaturated compounds. To find suitable ethenolysis conditions and to identify the possible products arising from the variety of different unsaturated fatty acids present in the crude algae oil, the individual fatty acid esters were subjected to ethenolysis as model substances.

The ethenolysis of methyl oleate leads to 1-decene (HC10:1) and methyl 9-decenoate (E10:1) as ethenolysis products (**Scheme 4-1**, x=7). In addition to the desired cross-metathesis products, two self-metathesis products, namely 9-octadecene (HC18:1) and dimethyl 9-octadecenedioate (DE18:1), can also be formed (**Scheme 4-1**, x=7).



Scheme 4-1: Self- and cross-metathesis products of methyl oleate and methyl palmitoleate, respectively, with ethylene.

Initially, a relative low ethylene pressure of 1.5 bar and dichloromethane as solvent was chosen. As a catalyst with a high kinetic selectivity for ethenolysis products, which performs poorly in self-metathesis1st generation Ru catalyst was selected. The ethenolysis of FA18:1, as an ester of one of the mono-unsaturated fatty acids present in algae oil from *P. tricornutum*, was conducted at 1.5 bar ethylene with HG1 as catalyst and a catalyst loading of 0.1 mol% and 0.5 mol%, respectively (**Table 4-1**). For 0.1 mol% catalyst loading a conversion of 63% and a selectivity for ethenolysis products of 80% were observed, while for 0.5 mol% 72% conversion and a selectivity of 65% for methyl 9-decenoate and 1-decene were found. For comparison, the same experiments were conducted with HG2 as catalyst (**Table 4-1**), which gave conversions of 61% and 72% (0.1 mol% and 0.5 mol%, respectively) and selectivities of 45% and 56%. As expected, the selectivities for ethenolysis products in the experiments with HG2 as catalyst are lower and the corresponding self-metathesis is more pronounced.[129] Additionally, the ethenolysis experiments with HG1 were carried out employing a higher pressure of 10 bar. As expected, the selectivities for ethenolysis products were enhanced significantly to 96% with a catalyst loading

of 0.1 mol% and to 97% with 0.5 mol%, respectively. Also, the conversions were improved. The best result was obtained with a high catalyst loading of 0.5 mol% at 10 bar. However, due to easier handling and no need of high-pressure equipment the following ethenolysis experiments were conducted at 1.5 bar using HG1.

Table 4-1: Ethenolysis of methyl oleate in dichloromethane.

Catalyst	Catalyst loading [mol%]	Ethylene pressure [bar]	Conversion[a] [%]	Selectivity[a] for ethenolysis products [%]
HG1	0.1	1.5	63	80
HG1	0.5	1.5	72	65
HG1	0.1	10	75	97
HG1	0.5	10	95	96
HG2	0.1	1.5	61	45
HG2	0.5	1.5	72	56

HG1: Hoveyda-Grubbs 1st generation catalyst, HG2: Hoveyda-Grubbs 2nd generation catalyst. Conditions: 2 mL (5.9 mmol) methyl oleate, 4 mL CH_2Cl_2, ambient temperature, 6h. [a] Determined via GC analysis.

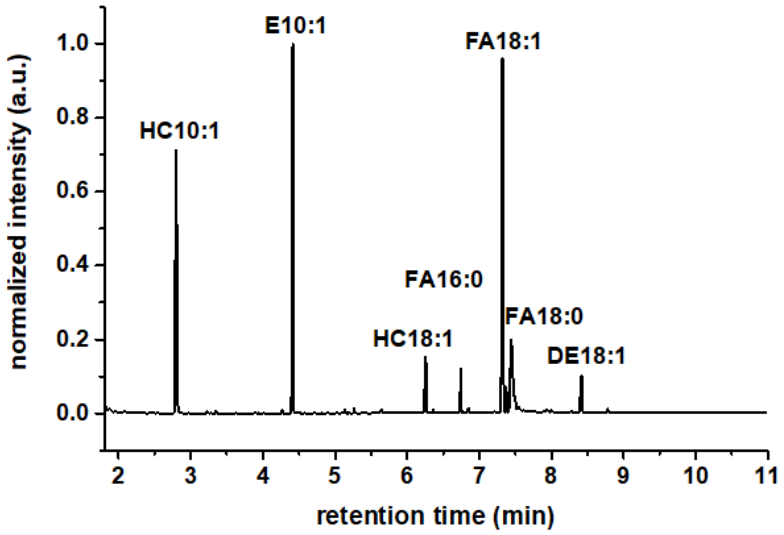


Figure 4-2: Gas chromatogram of the reaction mixture of ethenolysis of methyl oleate with assignment of the starting material methyl oleate (FA18:1), the ethenolysis products (methyl 9-decenoate (E10:1), 1-decene (HC10:1)) and self-metathesis products (9-octadecene (HC18:1), dimethyl 9-octadecenedioate (DE18:1)).

The ethenolysis of methyl palmitoleate, the methyl ester of the other mono-unsaturated fatty acid present in algae oil, leads to methyl 9-decenoate (E10:1) and 1-octene (HC8:1, **Scheme 4-1**, x=5). For the ethenolysis of methyl palmitoleate (FA16:1) 0.1 mol% HG1, 1.5 bar ethylene at ambient pressure and dichloromethane as solvent were applied. GC analysis (**Figure 4-3**)

revealed a conversion of 48% and a selectivity of 83% for ethenolysis products with 13% of self-metathesis products (dimethyl 9-octadecenedioate (DE18:1) and 7-tetradecene (HC14:1)). The selectivity is comparable to the corresponding experiment with methyl oleate, except for a slightly lower conversion, which might be due to difference in substrate purity.

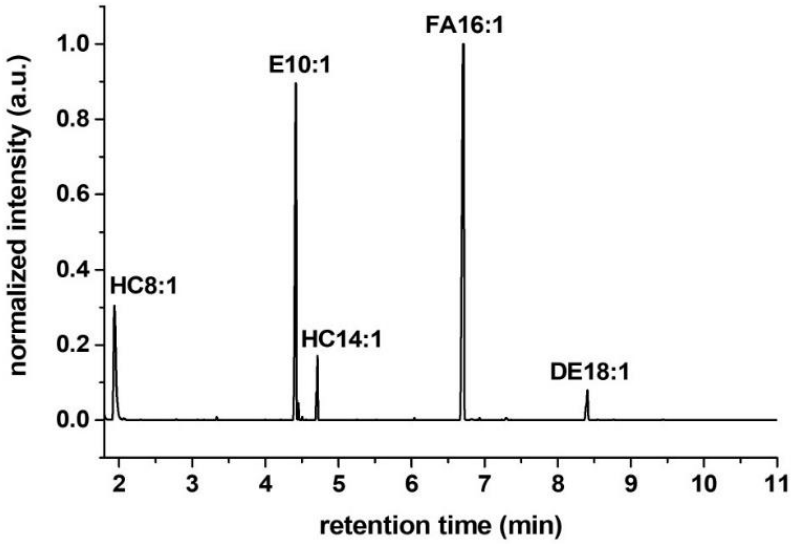
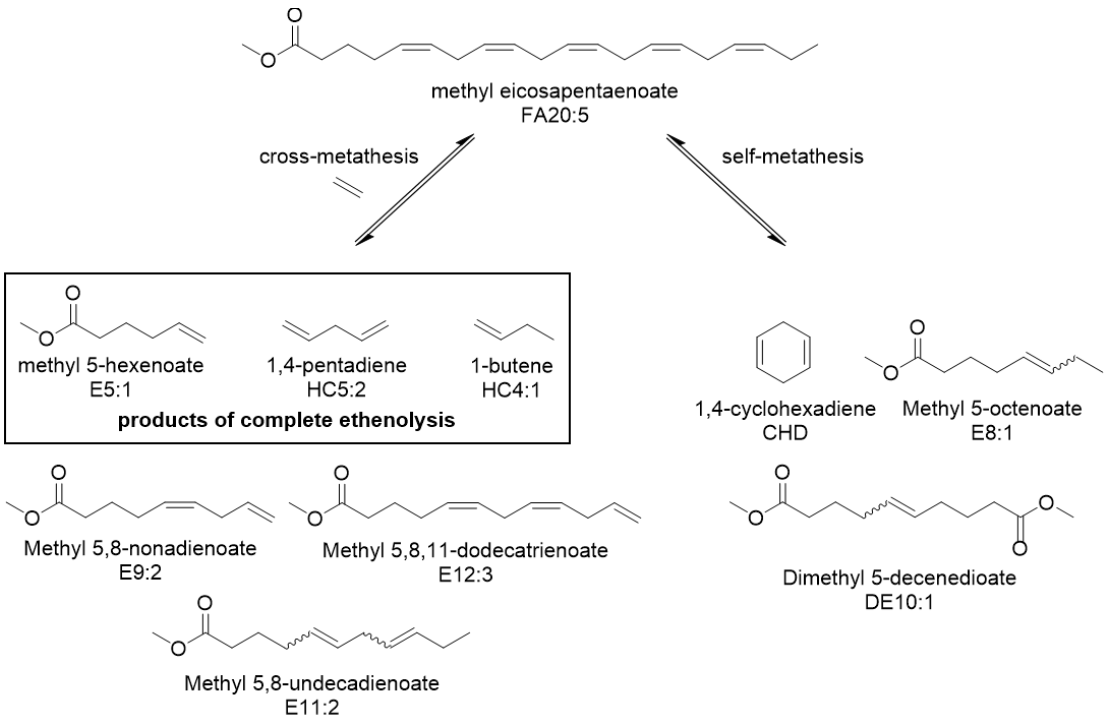


Figure 4-3: Gas chromatogram of the reaction mixture of ethenolysis of methyl palmitoleate with assignment of the starting material (methyl palmitoleate (FA16:1)), the ethenolysis products (methyl 9-decenoate (E10:1), 1-octene (HC8:1)) and self-metathesis products (7-teradecene (HC14:1), dimethyl 9-octadecenedioate (DE18:1)).

One unique component present in algae oil is the five-fold unsaturated fatty acid eicosapentaenoic acid. Ethenolysis of this poly-unsaturated fatty acid can give access to a range of versatile building blocks. In a complete ethenolysis of each double bond of methyl eicosa-pentaenoate (FA20:5), methyl 5-hexenoate (E5:1), 1-butene (HC4:1) and four equivalents of 1,4-pentadiene (HC5:1) can be formed (**Scheme 4-2**). Note, however, that HC5:2 and HC4:1 were not detected in the GC analysis protocol due to their low boiling point. 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. The suppression of this favoured intramolecular metathesis to 1,4-cyclohexadiene is the major challenge of cross-metathesis with FA20:5 as starting material.



Scheme 4-2: Ethenolysis and self-metathesis products of FA20:5.

With 0.1 mol% Hoveyda-Grubbs 1st generation catalyst per double bond at ambient temperature and 1.5 bar ethylene FA20:5 was completely converted as observed by GC analysis. However, the ethenolysis of all five double bonds was incomplete leading to the formation of multiple unsaturated products with internal double bonds (such as E9:2 or E12:3, see **Scheme 4-2**). Furthermore, it is possible that an ethenolysis step is followed by a self-metathesis or other cross-metathesis steps, leading again to an even broader product spectrum.

In this experiment the following ethenolysis products were observed (see **Scheme 4-2** and **Figure 4-4**): methyl 5-hexenoate (E5:1), methyl 5,8-nonadienoate (E9:2) and methyl 5,8,11-dodecatrienoate (E12:3) as well as the self-metathesis products 1,4-cyclohexadiene (CHD), methyl 5-octenoate (E8:1), methyl 5,8-undecadienoate (E11:2) and dimethyl 5-decenedioate (DE10:1). The selectivity was defined as the percentage share of methyl 5-hexenoate of all fatty acid esters formed, amounting to 41 %. By contrast to the butenolysis of FA20:5 in the cross-metathesis with ethylene additionally different self-metathesis products namely 1,4-cyclo-hexadiene (CHD), methyl 5-octenoate (E8:1), methyl 5,8-undecadienoate (E11:2) and dimethyl 5-decenedioate (DE10:1) are obtained. This can be explained by the more pronounced self-metathesis due to the lower stability of the methylidene complex in contrast to its ethylidene analogue formed in the butenolysis reaction (c.f. chapter 3.2.2). Furthermore, the concentration of ethylene is significantly lower than of 2-butene leading to more self-metathesis reactions. In the ethenolysis a 2-fold excess of ethylene was applied, whereas in the butenolysis a 10-fold excess of 2-butene was used.

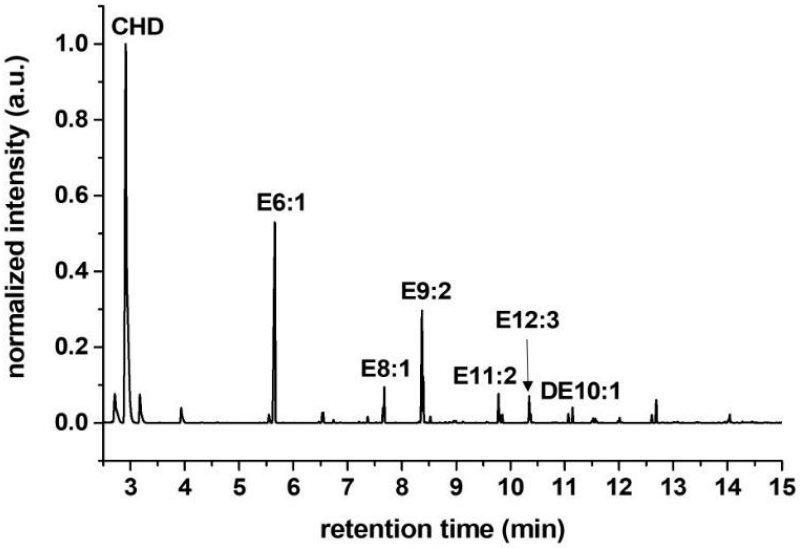


Figure 4-4: Gas chromatogram of the reaction mixture of ethenolysis of FA20:5 with assignment of identified cross-metathesis products (methyl 5-hexenoate (E6:1), methyl 5,8-nonadienoate (E9:2) and methyl 5,8,11-dodecatrienoate (E12:3)) and self-metathesis products (1,4-cyclohexadiene (CHD), methyl 5-octenoate (E8:1), methyl 5,8-undecadienoate (E11:2), and dimethyl 5-decenedioate (DE10:1)).

1,4-cyclohexadiene and methyl 5-hexenoate were identified via enrichment experiments with commercially available samples. The product identification of the poly-unsaturated products was complicated due to the large number of double bond isomers. For this, the crude product mixture was hydrogenated using Pd/C as catalyst and the obtained saturated compounds were analysed via GC (**Figure 4-5**). Via comparison of retention times and enrichment experiments with commercially available genuine samples the obtained saturated products could be identified. This allows for further identification of their unsaturated counterparts before hydrogenation. Note that cyclohexane with its lower boiling point is not observed in the gas chromatogram of the hydrogenated reaction mixture as the workup includes an evaporation step.

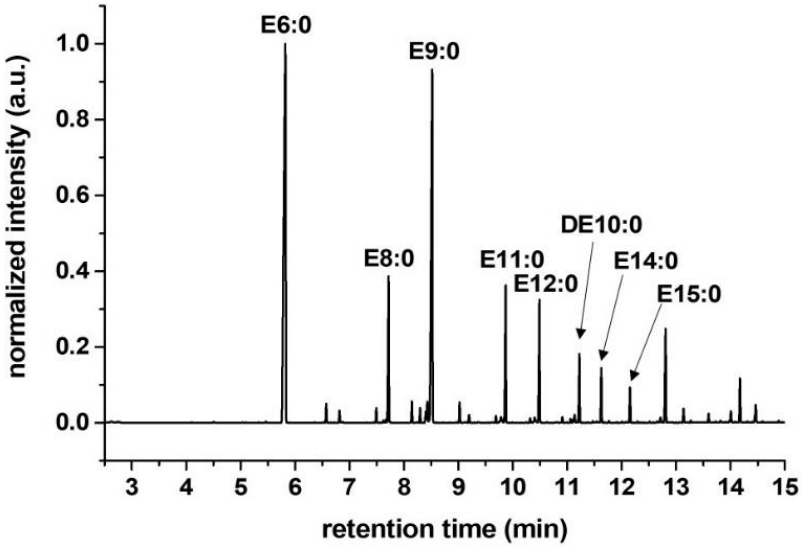


Figure 4-5: Gas chromatogram of the hydrogenated product mixture obtained from the ethenolysis of FA20:5 with assignment of formed compounds (methyl hexanoate (E6:0), methyl octanoate (E8:0), methyl nonanoate (E9:0), methyl undecanoate (E11:0), methyl dodecanoate (E12:0), dimethyl decanedioate (DE10:0), methyl tetradecanoate (E14:0), methyl pentadecanoate (E15:0)).

With the ethenolysis products and self-metathesis products of FA16:1, FA18:1 and FA20:5 identified, ethenolysis was performed on extracted algae oil from the microalgae *P. tricornutum*. The oil used was extracted in the stationary phase via a modified Folch method and analysed via GC. In accordance to the experiments of the single fatty acid esters as model substrates, the ethenolysis of the crude algae oil was performed using 0.1 mol% Hoveyda-Grubbs 1st generation catalyst precursor (per double bond) at 1.5 bar ethylene pressure and ambient temperature. The reaction mixture was transesterified with MeOH and analysed via GC (**Figure 4-6**). For the mono-unsaturated fatty acid esters (FA16:1 and FA18:1) conversions of 67% and 69%, respectively, were observed. These are comparable to the conversions obtained for the ethenolysis of the single fatty acid esters under the same conditions. Different from the model substrate, FA20:5 in the algae oil was not complete converted (89%). Selectivity for the ethenolysis products of the mono-unsaturated compounds are 83% for FA16:1 and 87% for FA18:1 and in the same range as for the model substrates. The selectivity for methyl 5-hexenoate as ethenolysis products of FA20:5 was found to be 55%.

Minor unassigned signals in the GC trace of the ethenolysis of algae oil possible arise from components present in the starting algae oil, side-products from ethyl vinyl ether quenching or further cross-metathesis products. As expected, the content of saturated fatty acids, myristic and palmitic acid, remained unaltered during the metathesis reaction.

All in all, the used catalyst is compatible with the various components of algae oil and converts the unsaturated long chain fatty acids to a range of olefins and unsaturated acids with the same conversions and selectivities as for the model substrates (at the catalyst loading studied).

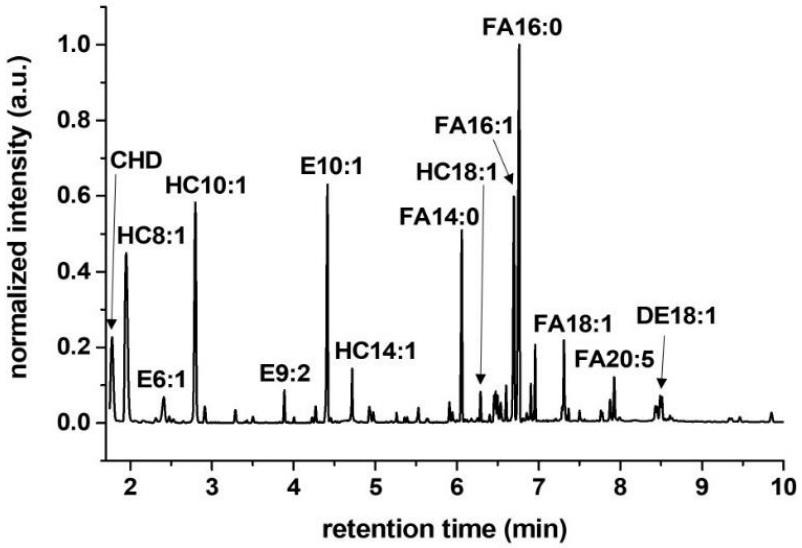


Figure 4-6: Gas chromatogram of the reaction mixture of ethenolysis of crude algae oil after transesterification with methanol. Assignment of identified cross-metathesis products (1-octene (HC8:1), methyl 5-hexenoate (E6:1), 1-decene (HC10:1), methyl 5,8-nonadienoate (E9:2) and methyl 9-decenoate (E10:1)) and self-metathesis products (1,4-cyclohexadiene (CHD), 7-tetradecene (HC14:1), 9-octadecene (HC18:1), dimethyl 9-octadecenedioate (DE18:1)), unconverted starting materials (FA16:1, FA18:1 and FA20:5) and saturated fatty acid methyl esters (FA14:0 and FA16:0).

4.2.2 Isomerizing Ethenolysis of Methyl Oleate

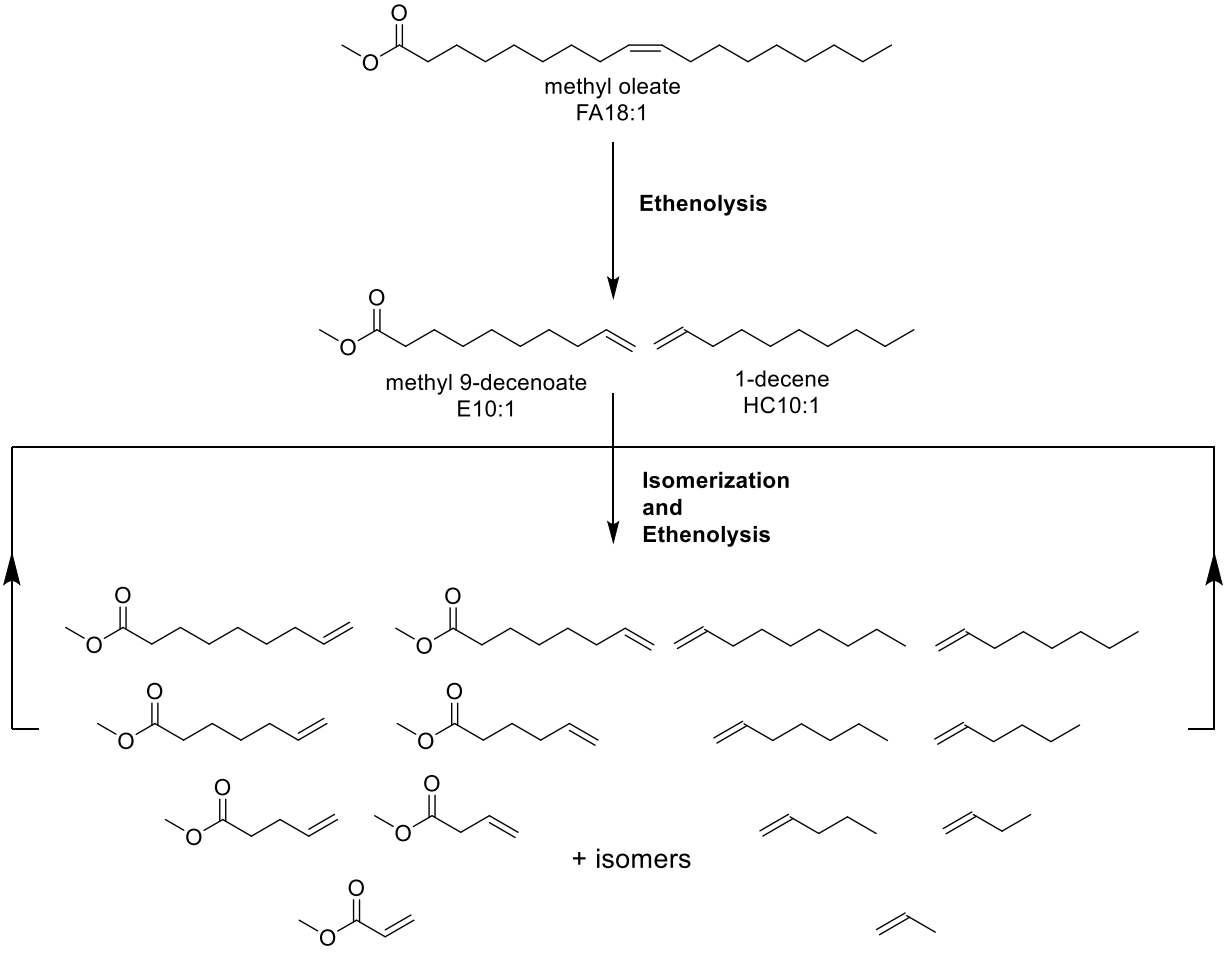
The product scope accessible by ethenolysis of fatty acids is restricted by the variety of fatty acids occurring in nature. However, the product spectrum of the metathesis reaction can be increased by isomerization of the internal double bond(s).

Isomerization generally occurs in metathesis reactions catalyzed by Ru alkylidene complexes to some extent. The reasons for this often undesired side-reaction are Ru hydride complexes formed by decomposition of the metal alkylidene.[37, 38] This behaviour is even more pronounced for the second generation catalysts compared to the first generation catalysts. Yet, to achieve a high isomerization activity relative harsh conditions and suitable solvents are necessary.

Rather, in this work an additional isomerization catalyst is used for the isomerizing ethenolysis to achieve control and defined conditions. Thus, the isomerization can be induced at a defined stage of the overall reaction scheme which is an important point in this concept. This work focusses on the formation of selective products with chain length below 10 carbon atoms

starting from methyl oleate, which can be accessible by a first chain shortening ethenolysis step and subsequently induced isomerizing ethenolysis.

The first step of this approach comprises an ethenolysis reaction, ideally with full conversion, and without at this point unwanted side reactions such as self-metathesis or isomerization. In case of methyl oleate as starting material, the chain length is shortened from 18 carbon atoms to 10 carbon atoms. In the following isomerization step the double bonds of the obtained ethenolysis products, namely 1-decene and methyl 9-decenoate, are continuously converted into equilibrium mixtures of double bond isomers, which concurrently undergo ethenolysis leading to products of even shorter chain lengths. Both steps, ethenolysis and isomerization, can proceed multiple times leading to further shortening of the chain length and ending up ultimately at C3 products, namely propene and methyl acrylate. Assuming that in the first ethenolysis step a full conversion is achieved and self-metathesis is effectively suppressed throughout the entire reaction process, a distribution of unsaturated products in case of methyl oleate with chain lengths shorter than 10 carbon atoms can be obtained (**Scheme 4-3**). These short and mid-chain 1-olefins from fatty acids resemble key intermediates and basic chemicals of today's petrochemical industry.



Scheme 4-3: Isomerizing ethenolysis of methyl oleate leading to a product mixture with chain lengths shorter than 10 carbon atoms.

In the following isomerizing ethenolysis experiments [Pd(dtbpx)(OTf)$_2$] was chosen as isomerization catalyst precursor, as this catalyst system is known to be compatible with fatty acids from plants or microalgae and the underlying mechanism is elucidated.[68, 128] Initially, Hoveyda-Grubbs 1st generation catalyst was chosen as metathesis catalyst, as with this catalyst good results in the ethenolysis of methyl oleate (see chapter 4.2.1) were observed. To investigate the compatibility of these two orthogonal catalysts, methyl oleate was used as starting material in this two-step concept, while the progress of reaction over time was monitored via GC (**Figure 4-7**).

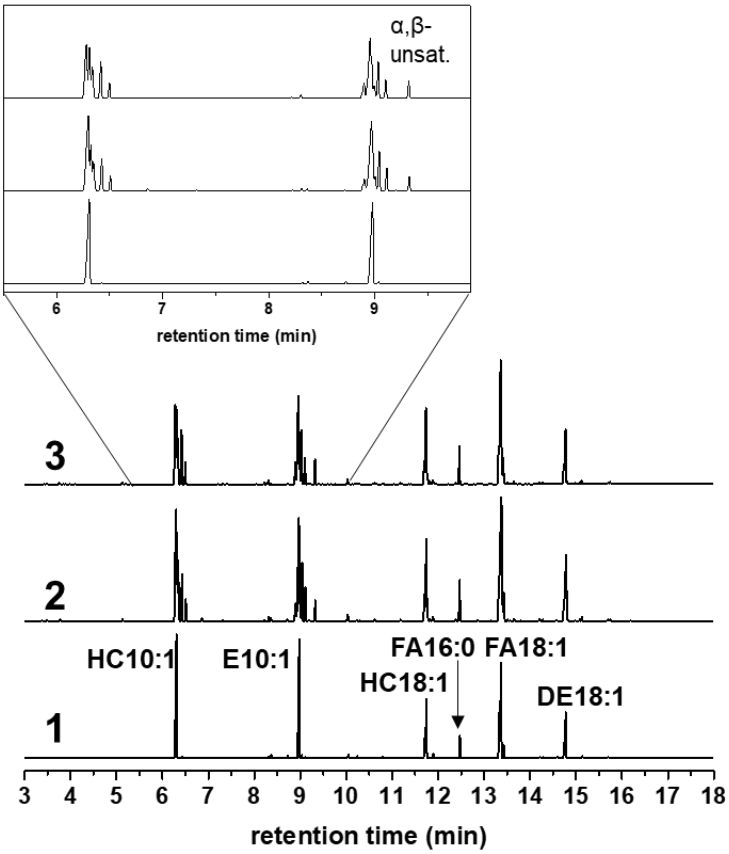


Figure 4-7: Gas chromatograms of the reaction mixture. 1 (bottom): after 3 hours of ethenolysis (0.5 mol% HG1, 1.5 bar ethylene, ambient temperature, 4 mL CH_2Cl_2) with assignment of ethenolysis products (1-decene (HC10:1), methyl 9-decenoate (E10:1)), self-metathesis products (HC18:1 9-octadecene, DE18:1 dimethyl 9-octadecenedioate) and the starting material methyl oleate FA18:1. 2 (center): 1.5 h after the addition of 0.5 mol% [Pd(dtbpx)(OTf)$_2$] dissolved in 1 mL MeOH 3 (top): 3 h after the addition of [Pd(dtbpx)(OTf)$_2$].

In order to split the hydrocarbon chain, the fatty acid ester was converted in an ethenolysis reaction with 0.5 mol% Hoveyda-Grubbs 1st generation dissolved in CH_2Cl_2 and 1.5 bar ethylene at ambient temperature for 3 hours (**Figure 4-7**, bottom). Subsequently, 0.5 mol% of the isomerization catalyst [Pd(dtbpx)(OTf)$_2$] dissolved in MeOH was added. After another 3 hours the reaction was quenched with ethyl vinyl ether (**Figure 4-7**, top). The conversions and selectivities were determined over FA16:0 as internal GC standard. Surprisingly, the conversion of 72% does not change any more after the addition of the Pd catalyst and also the selectivity (66%) for the

ethenolysis products remains constant. All in all, after the addition of the isomerization catalyst no more metathesis seems to occur. Yet, the isomerization catalyst is active as indicated by additional signals arising in the GC. This becomes particularly obvious for the shorter chain metathesis products (HC10:1 and E10:1), where the signals of the single isomers are more separated than for the longer products (HC18:1 and DE18:1). In the case of E10:1 the α,β unsaturated isomer is observed as single signal at higher retention time than the other isomers (**Figure 4-7**, center). Furthermore, the isomerization seems to be ongoing even after 3 hours, as the ratio of the signals of the single isomers is still changed (**Figure 4-7**, center and top).

For identification of the isomers genuine samples of 1-decene and methyl 9-decenoate were isomerized by addition of [Pd(dtbpx)(OTf)$_2$] with methanol in order to compare the retention times (**Figure 4-8**). In both cases the obtained signal pattern is similar to the once obtained in the isomerizing ethenolysis after addition of isomerization catalyst (**Figure 4-7**). Note that already in the genuine sample some isomers are observed as impurity to a certain extent.

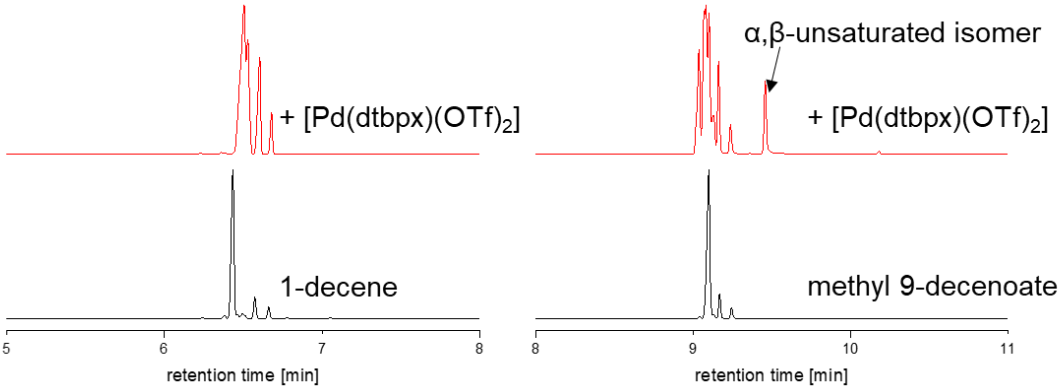


Figure 4-8: Gas chromatograms of commercial available samples of 1-decene and methyl 9-decenoate (bottom, black) and the corresponding isomerized samples (top, red) in MeOH after 1 h at 50°C.

Possible reasons that no further conversion in metathesis is observed might be the limited stability of HG1 over time or deactivation by addition of [Pd(dtbpx)(OTf)$_2$].

In order to further investigate the impact of the isomerization catalysts on the activity of HG1, ^{31}P-NMR spectra of the individual catalyst precursors and a mixture thereof were recorded (**Figure 4-9**). For both single catalysts sharp signals were observed (**Figure 4-9**, spectrum 1 and 2). However, upon mixing, the phosphorous signals of the Pd-H complex[125, 128] broaden slightly, while the signal of the Ru metathesis catalyst seems not to be effected (**Figure 4-9**, spectrum 3). After another 2 hours the signal of HG1 decreases and additional signals appear which could originate from decomposition products of the metathesis catalyst such as protonated and/or (partly) oxidized phosphines (**Figure 4-9**, spectrum 4).

Yet, these observations do not fully explain the complete deactivation of HG1 after the addition of [Pd(dtbpx)(OTf)$_2$] in the above catalysis experiment.

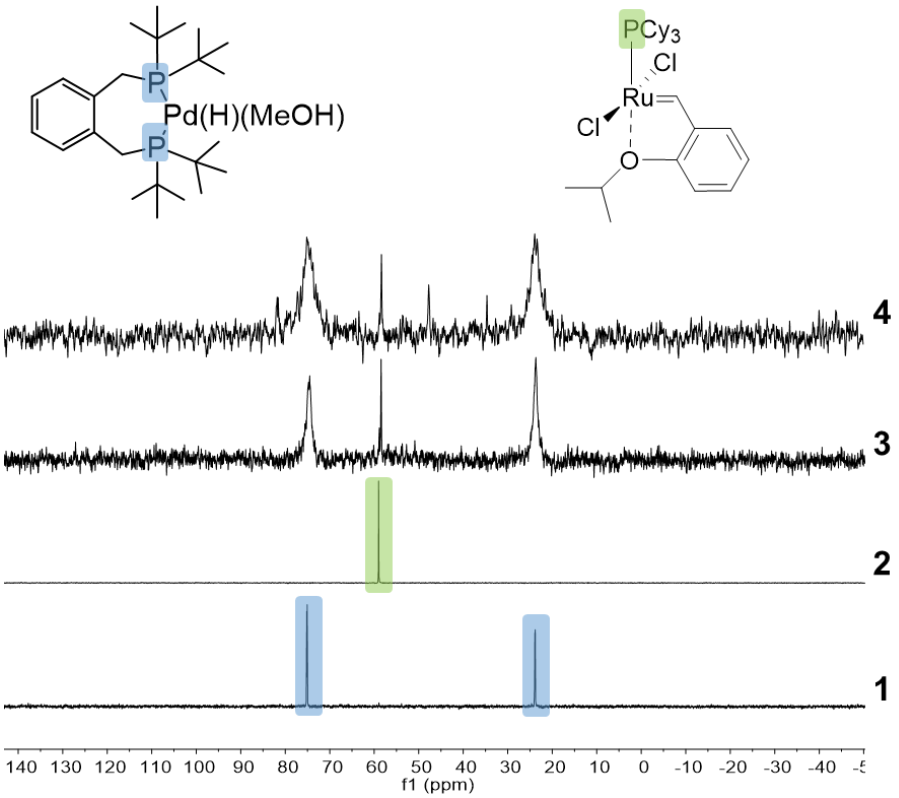


Figure 4-9: ^{31}P-NMR spectra of 1: 55 µmol [Pd(dtbpx)(OTf)$_2$] dissolved in CD_2Cl_2 and MeOH; 2: 8 µmol Hoveyda-Grubbs 1st generation catalyst dissolved in CD_2Cl_2; 3: mixture of 1 and 2; 4: mixture after 2 hours.

Although selectivity for ethenolysis is lower for HG2 compared to HG1 as shown in the results before (see chapter 4.2.1), HG2 was investigated in the isomerising ethenolysis as HG2 is known for a higher robustness and activity due to the lower kinetic lability of the NHC-ligand compared to the phosphine ligand of HG1.

To obtain a mixture of products with chain length shorter than ten carbon atoms selectively the first ethenolysis step should be complete and in particular have a high selectivity for ethenolysis products. To meet these demands, the conditions of the first ethenolysis step with HG2 as catalyst were adjusted. First, the concentration of the starting material was decreased which has a beneficial effect on the selectivity. Furthermore, due to the higher thermal stability of HG2, a higher reaction temperature of 60 °C could be applied. This increase in temperature has a positive impact on the catalyst activity. However, for this purpose another solvent with a higher boiling point had to be chosen, in this case toluene instead of dichloromethane was applied.

These conditions were further applied in the first step of isomerizing ethenolysis. After 2 hours, a conversion of 82% and selectivity for ethenolysis products of 76% were observed (**Figure 4-10**, bottom). Subsequently, 0.8 mol% of the isomerization catalyst [Pd(dtbpx)(OTf)$_2$]

dissolved in MeOH was added. Thereafter again no further metathesis activity was observed, but the unsaturated compounds were subject to isomerization (**Figure 4-10**, center). Obviously, in accordance to the results observed for HG1, the isomerizing catalyst used also leads to decomposition of HG2. In order to convert the isomerized products in ethenolysis and thus to obtain the desired product mixture, further metathesis catalyst (1.2 mol%) was added. GC analysis shows a broad spectrum of unsaturated products (**Figure 4-10**, top) indicating a successful isomerizing ethenolysis overall. The conversion of methyl oleate reached 97%.

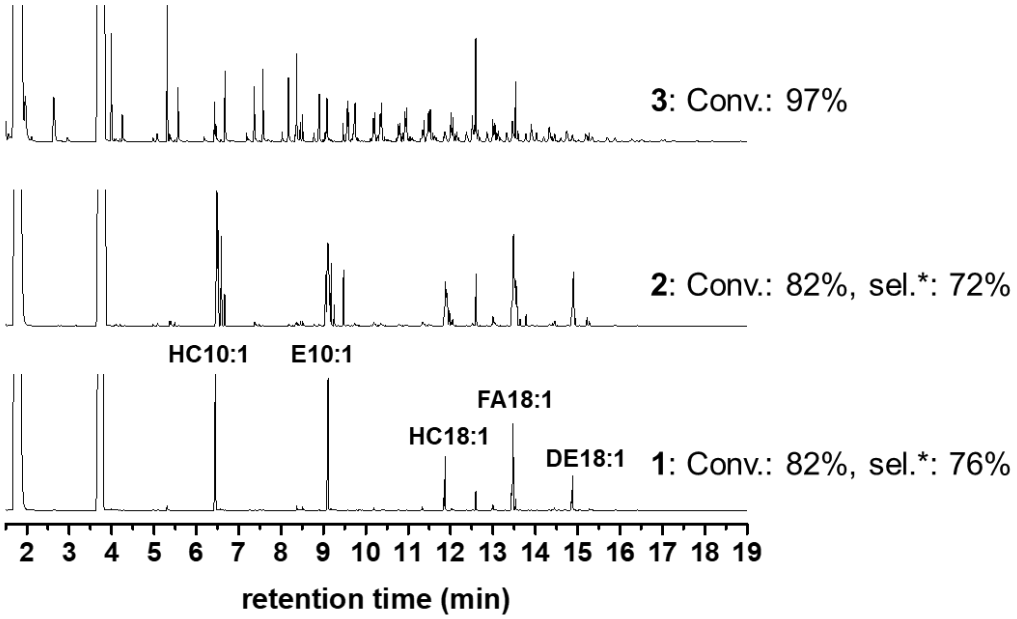


Figure 4-10: Gas chromatograms of the reaction mixture 1: after 2 hours of ethenolysis (0.2 mol% HG2, 1.5 bar ethylene, 60 °C) with assignment of ethenolysis products (1-decene HC10:1, methyl 9-decenoate), self-metathesis products (HC18:1 9-octadecene, DE18:1 dimethyl 9-octadecenedioate) and the starting material methyl oleate FA18:1. 2: 1 h after the addition of 0.8 mol% [Pd(dtbpx)$(OTf)_2$] 3: 1 h after the addition of 1.2 mol% HG2. *Selectivity for the ethenolysis products methyl 9-decenoate E10:1 and 1-decene including their double bond isomers.

For analysis of the selectivity and product distribution the various products were divided into four groups (**Figure 4-11**). The first group (**Figure 4-11**, green box) includes the primary ethenolysis products methyl 9-decenoate and 1-decene as well as their corresponding isomers. The primary self-metathesis products 9-octadecene (HC18:1) and dimethyl 9-octadecenedioate (DE18:1) and their isomers form the next group (**Figure 4-11**, yellow box). The most desired group contains all products of isomerizing ethenolysis and metathesis with a chain length below 10 carbon atoms (**Figure 4-11**, blue box), which should be ideally the largest part. Also, all products with a chain length of C>10 are grouped (**Figure 4-11**, red box).

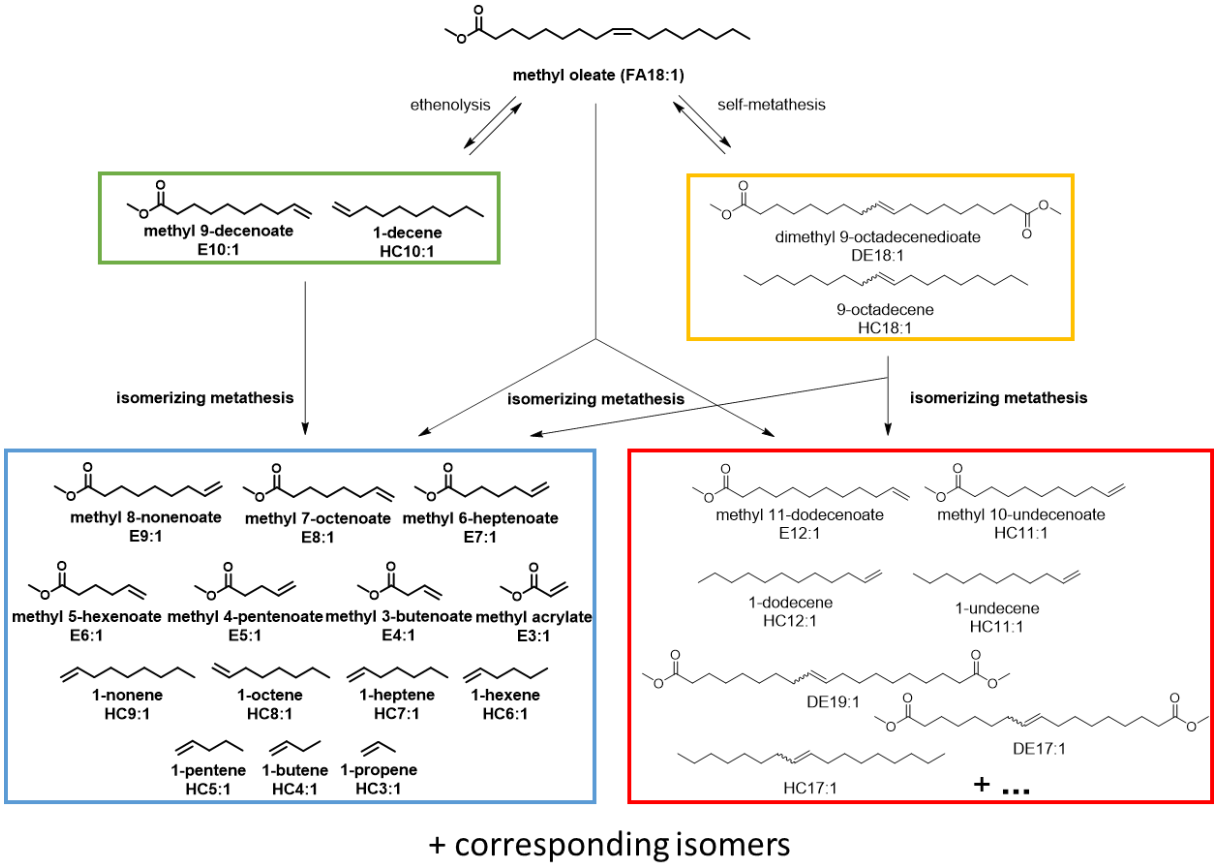


Figure 4-11: Possible products from isomerizing ethenolysis: : primary ethenolysis products (green box), primary self-metathesis products (yellow box), products of isomerization and metathesis C<10 (blue box), products of isomerization and metathesis C>10 (red box).

For identification of the different reaction products genuine samples of alkenes with chain lengths of C6-C12 as well as unsaturated methyl esters (C3-C11) were isomerized with [Pd(dtbpx)(OTf)$_2$] in methanol and analysed via GC (see experimental section, **Figure 4-15** and **Figure 4-16**). Based on comparison of the retention times the assignment of the different products is given in **Figure 4-12**.

All in all, about 10 mol% (10 wt%) primary ethenolysis products as well as 3 mol% (6 wt%) primary self-metathesis products remain in the final reaction mixture. The ratio of the products of isomerizing metathesis with a chain length of C<10 amounts to 37 mol% (29 wt%), whereas 50 mol% (55 wt%) of the reaction mixture are products of isomerization and metathesis with chain length of more than 10 carbon atoms.

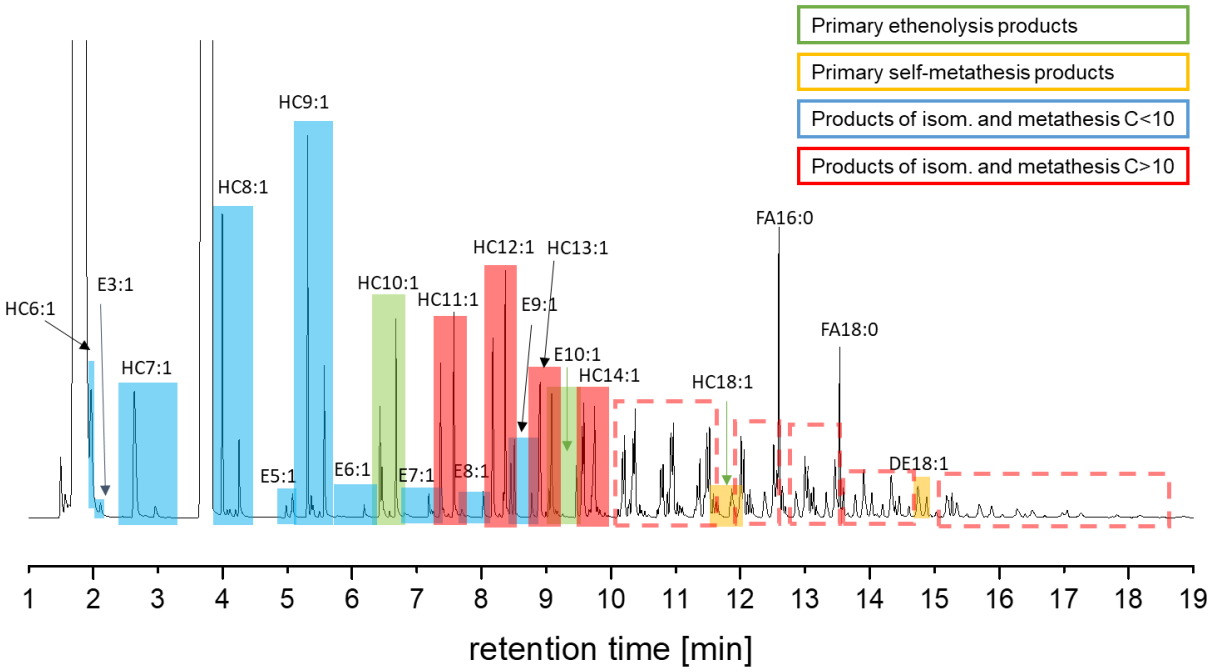


Figure 4-12: Gas chromatogram of the isomerizing ethenolysis of methyl oleate and assignment of the different products and products groups. The signals marked with a dashed line are assumed to belong to the products of isomerization and metathesis C>10.

Please note, that products of isomerization and metathesis with a chain length of C<10 (blue box) are underestimated as hydrocarbons with a chain length shorter than 6 carbon atoms are not detected by the GC procedure used. Furthermore, during work-up of the crude reaction mixture a characteristic smell of acrylate was observed, which could indicate a loss of this product and lead to a further underestimation of products (and other volatilities) of isomerizing metathesis with C<10.

Furthermore, it has to be mentioned, that each area in the gas chromatogram corresponding to alkene isomers with a certain chain length is dominated by two major signals (**Figure 4-12**). As indicated by GC analysis of different isomers of octene for example, the signal with the lower retention time can be ascribed to the corresponding 1-olefine while the other is assumed to be the 2-olefin. In another experiment under same reaction conditions but a catalyst loading of 0.4 mol% in the first ethenolysis step, a similar chain length distribution is obtained. However, in the gas chromatogram of this reaction mixture for each alkene with a given chain length more signals and thus more isomers were observed (**Figure 4-13**).

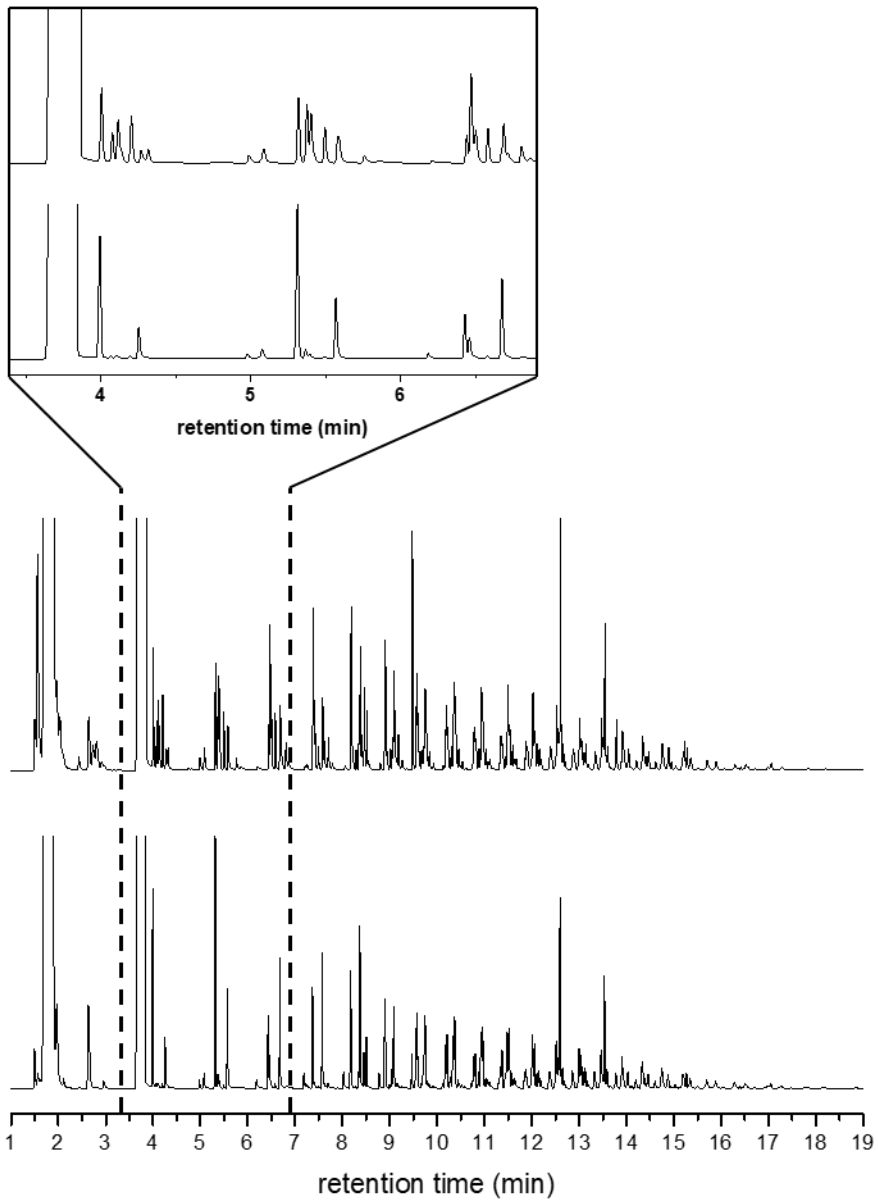


Figure 4-13: Gas chromatograms of isomerizing ethenolysis of methyl oleate applying 0.2 mol% (bottom) or 0.4 mol% (top) HG2 in the first ethenolysis step.

All in all, the isomerizing ethenolysis with the commercially available Ru catalysts HG2 and [Pd(dtbpx)(OTf)$_2$] as additional isomerization catalyst is feasible, but leave room for improvement. The influence of the two catalysts on each other should be further investigated and the conditions for the ethenolysis as well as isomerization should be optimized. Ideally, this concept can be applied on fatty acids from microalgae to obtain short- and mid-chain unsaturated products which are useful for example as building blocks for polymers.

4.3 Conclusion

Ethenolysis is a very useful transformation for upgrading fatty acids/esters from microalgae as renewable feedstock to terminal unsaturated products. These products are valuable intermediates for a broad field of applications such as fuels, lubricants, waxes or surfactants. With the help of an additional isomerization step in combination with ethenolysis, an even broader product spectrum is accessible.

To find suitable ethenolysis conditions and to identify the possible products arising from the variety of different unsaturated fatty acids present in the crude algae oil, the individual fatty acid esters were subjected to ethenolysis as model substances. Applying 0.1 mol% 1st generation Hoveyda-Grubbs catalyst and 1.5 bar ethylene at ambient temperature for 6 h conversions of 48% and 63%, respectively, were observed for the mono-unsaturated fatty acid methyl esters FA16:1 and FA18:1 with a selectivity for ethenolysis products of about 80%. Under the same conditions the FA20:5 was completely converted. However due to incomplete ethenolysis of all five double bonds and favoured intramolecular self-metathesis leading to 1,4-cyclohexadiene a complex mixture of multiple unsaturated products is obtained. With the ethenolysis products and self-metathesis products of FA16:1, FA18:1 and FA20:5 identified, ethenolysis was performed on extracted algae oil from the microalgae *P. tricornutum* applying the conditions described above. For the mono-unsaturated fatty acids present in the oil conversions of 67% and 69%, for FA16:1 and FA18:1, respectively, were observed with selectivities up to 87%. The conversion of the five-fold unsaturated fatty acid was determined to 89%. All in all, it was demonstrated that HG1 catalyst is compatible with the various components present in microalgae oil and converts the unsaturated fatty acids with the same conversions and selectivities as for the model substrates.

The product scope accessible by ethenolysis of fatty acids is limited in principle and depends on the chain length of the starting material and the position(s) of the double bond(s). Yet, the product spectrum can be further increased by a tandem reaction combining ethenolysis with an additional double bond isomerization step. Starting from methyl oleate as model substrate this work focusses on the selective formation of products with chain length below 10 carbon atoms. This is achieved by a first ethenolysis step, which splits the fatty acid hydrocarbon chain selectively leading to 1-decene (HC10:1) and methyl 9-decenoate (E10:1) if no side reaction occurs. Subsequently, the isomerization is induced by an additional catalyst and the primary ethenolysis products are continuously converted into equilibrium mixtures of double bond isomers, which concurrently undergo ethenolysis leading to products of even shorter chain lengths. This two-step concept was demonstrated with methyl oleate as model substrate applying HG2 as metathesis catalyst and [Pd(dtbpx)(OTf)$_2$] as additional isomerization catalyst resulting in a broad product distribution (**Figure 4-14**).

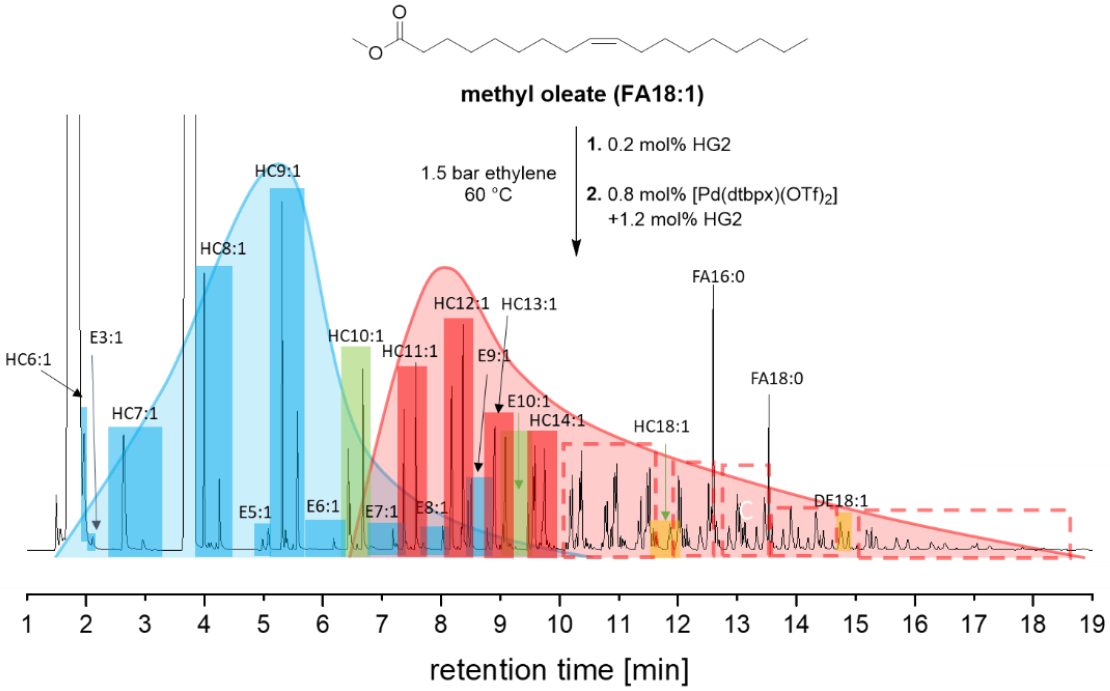


Figure 4-14: Gas chromatogram of the isomerizing ethenolysis of methyl oleate and assignment of the different products. Products of isomerization and metathesis C<10 are marked in blue, products of isomerization and metathesis C>10 are marked in red.

In total, about 37 mol% of the desired products of isomerizing ethenolysis with a chain length C<10 were found. Besides this about 10 mol% primary ethenolysis products as well as 3 mol% primary self-metathesis products were observed in the final reaction mixture. Whereas still 50 mol% of the reaction mixture are products of isomerization and metathesis with chain length of more than 10 carbon atoms.

4.4 Experimental Section

4.4.1 General Methods and Materials

All catalyst precursors were used as received and stored in a glovebox under a nitrogen atmosphere. All reactions were conducted under inert gas atmosphere (argon or nitrogen) using standard glovebox or Schlenk techniques. Dichloromethane was dried over CaH_2 and distilled under a nitrogen atmosphere. Methanol was dried over magnesium and distilled under a nitrogen atmosphere. Toluene was dried with sodium, distilled and stored under an inert gas atmosphere. Ethylene (N4.5) was supplied by Air Liquide. Methyl oleate (92.5 %) kindly donated by DAKO AG was distilled under a nitrogen atmosphere prior to use. Methyl palmitate, methyl palmitoleate and methyl myristate were purchased from Nu-Check Prep, Inc. Methyl palmitoleate was purified by Kugelrohr distillation. Methyl eicosapentaenoate purchased from Carbosynth was distilled under vacuum and stored under inert atmosphere. Hoveyda-Grubbs 2nd generation was purchased from Carbosynth. Hoveyda-Grubbs 1st generation catalyst was purchased from Sigma-Aldrich. [Pd(dtbx)$(OTf)_2$] was prepared as reported previously.[122]

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}. Method 1: The injector temperature was 300 °C and the detector temperature 280 °C. The oven was kept at 50 °C for 3 min, then heated with 20°C min^{-1} to 280 °C, and kept isothermal at 280 °C for 5 min. Method 2: The oven was kept for 1 min at 90 °C, then heated with 30 °C min^{-1} to 280 °C, kept at 280 °C for 8 min., with an injector temperature of 300 °C and a detector temperature of 280 °C. All free acids were esterified with methanol and a catalytic amount of sulfuric acid prior to GC analysis.

NMR spectra were recorded on a Varian Unity Inova 400. ^{1}H NMR spectra were referenced to residual protonated solvent signals, ^{31}P NMR spectra to external 85% H_3PO_4.

4.4.2 Algae Cultivation and Extraction

The examined strain is a single clone colony of *Phaeodactylum tricornutum* (*P. tricornutum*) wildtype UTEX 646 (WT4). The algae were cultivated in 10 L flasks continuously in modified f2 cultivation medium[127] with artificial half concentrated sea salts (16.6 g L^{-1}, tropic marine) aerated with sterile ambient air in a day/night rhythm of 16/8 hours at 20 °C and with a light intensity of 35 µmol x s^{-1} x m^{-2}.

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 or a Contifuge® Stratos with a titanium rotor (5000 g, 4 °C) from Hereaus. The second centrifugation step was conducted with an Allegra 25R centrifuge (5000 g, 10 min at 4 °C) from Beckman coulter. The obtained cell pellet was stored at -28 °C.

The oil was extracted via a modified method of Folch *et al.*[70] The cell pellet was thawed and diluted with H_2O. The mixture was put on ice and pre-treated by ultrasonication for 10 min with a pulse of 10 s and amplitude of 60%, using an ultrasound homogenizer HD3200 from BANDELIN with a KE76 sonotrode. A mixture of chloroform and methanol (2:1) was added to establish a ratio of $CHCl_3$:MeOH:ddH2O = 8:4:3. The natural water content is supposed to be approximately 75%. The organic phase containing the lipids was collected and dried over $MgSO_4$. After removal of the solvent under reduced pressure the crude algae oil was obtained.

4.4.3 General Procedure for Ethenolysis Experiments

The ethenolysis experiments of methyl oleate, methyl palmitoleate, methyl eicosapentaenoate or algae oil in dichloromethane as solvent at an ethylene pressure of 1.5 bar were performed in a Schlenk flask. For ethenolysis the following amount of starting material and solvent were used: 2 mL (5.9 mmol) methyl oleate and 4 L dichloromethane; 0.5 mL (1.5 mmol) methyl palmitoleate and 1 mL dichloromethane, 1 mL (2.9 mmol) methyl eicosapentaenoate and 2 mL dichloromethane, or 0.9 g algae oil (which corresponds to 3 mmol double bonds) and 3 mL dichloromethane, respectively. The Schlenk flask was charged with a fatty acid ester or the algae oil under a nitrogen atmosphere. The flask was evacuated and then pressurised with 1.5 bar of ethylene. The reaction was initiated by the addition of a freshly prepared solution of the appropriate amount of the catalyst in dichloromethane and then stirred at room temperature. The reaction was quenched with ethyl vinyl ether.

Ethenolysis experiments of methyl oleate in dichloromethane as a solvent at 10 bar 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.

Products from the reaction of fatty acid methyl esters were directly analysed by GC after dilution with dichloromethane while the products from reactions with algae oil were

transesterified by heating over 3 days with MeOH and a catalytic amount of concentrated sulphuric acid prior to GC analysis.

4.4.4 Isomerizing Ethenolysis of Methyl Oleate

Isomerizing Ethenolysis with Hoveyda-Grubbs 1st Generation Catalyst

A Schlenk flask under a nitrogen atmosphere was charged with 2 mL (5.9 mmol) methyl oleate. The flask was evacuated and then pressurised with 1.5 bar of ethylene. Ethenolysis was initiated by the addition of a freshly prepared solution of HG1 (18 mg, 0.03 mmol, 0.5 mol%) in 4 mL CH_2Cl_2 and the mixture was stirred at room temperature. After 3 hours 0.5 mol% (23.6 mg, 0.03 mmol) [Pd(dtbpx)$(OTf)_2$] dissolved in 1 mL methanol was added. After a total reaction time of 6 hours the reaction was quenched by adding ethyl vinyl ether.

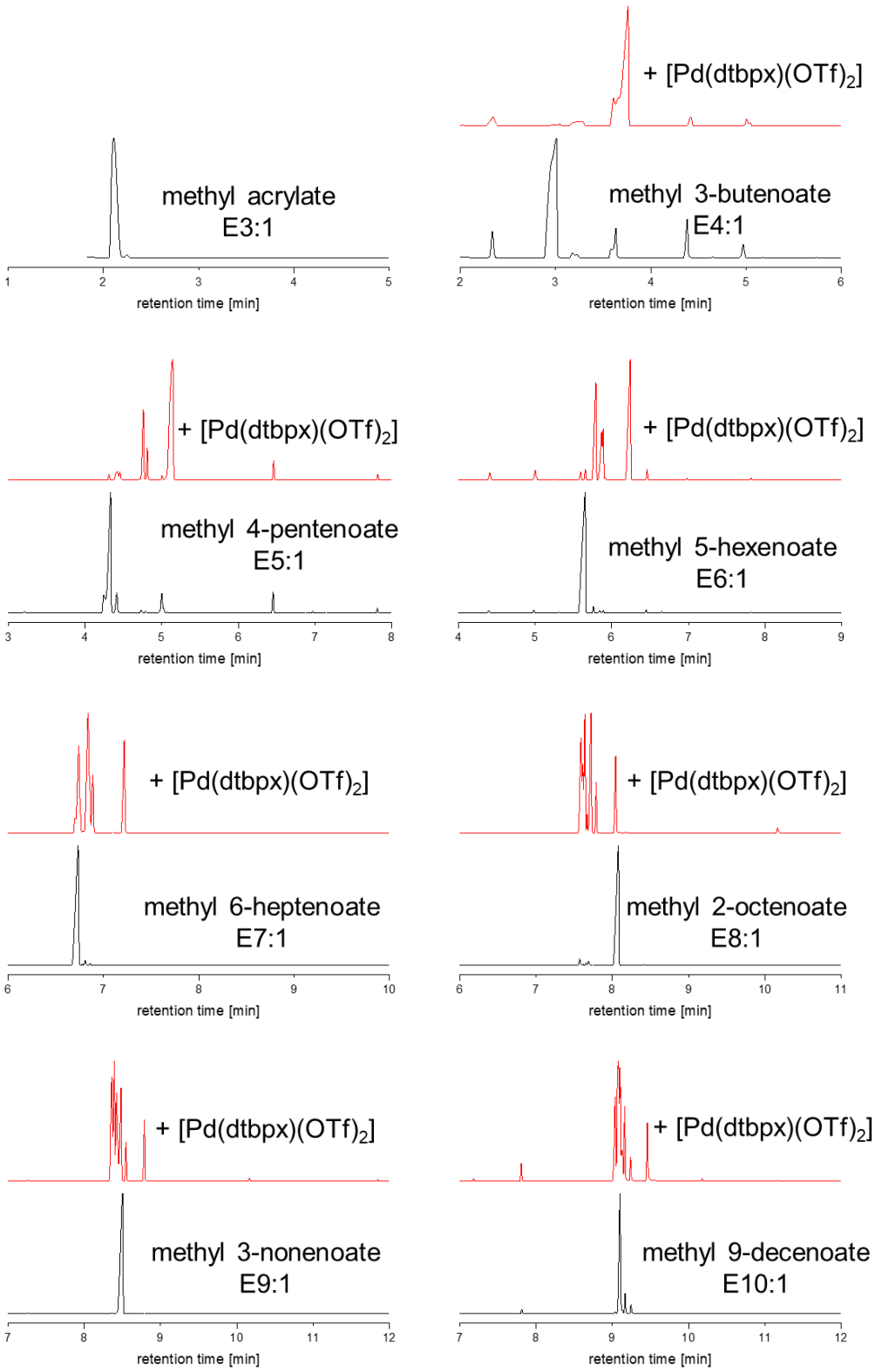
Isomerizing Ethenolysis with Hoveyda-Grubbs 2nd Generation Catalyst

A Schlenk flask under a nitrogen atmosphere was charged with 0.5 mL (1.5 mmol) methyl oleate. The flask was evacuated and then pressurised with 1.5 bar of ethylene. Ethenolysis was initiated by the addition of a freshly prepared solution of 1.8 mg (0.003 mmol) of Hoveyda-Grubbs 2nd generation in 4 mL toluene and the mixture was stirred at 60 °C. After 2 hours 9.4 mg (0.012 mmol) [Pd(dtbpx)$(OTf)_2$] dissolved in 0.5 mL methanol was added. After a total reaction time of 3 hours again Hoveyda-Grubbs 1st generation (11 mg, 0.018 mmol) dissolved in 1 mL toluene was added.

NMR Scale Studies of Interactions of Catalyst Precursors

All solid substrates were weighed into an NMR tube in a glove box and dissolved in the appropriate solvent. Hoveyda-Grubbs 1st generation catalyst (5 mg, 0.008 mmol) was transferred in an NMR tube and diluted in 0.5 mL CD_2Cl_2. Subsequently the NMR tube was closed with a septum. In an additional NMR tube 44 mg (0.055 mmol) [Pd(dtbpx)$(OTf)_2$] were dissolved in 0.3 mL CD_2Cl_2. After recording ^1H and ^{31}P $\{^1H\}$ NMR spectra for both samples, 0.4 mL MeOH was added to the sample with dissolved [Pd(dtbpx)$(OTf)_2$] and ^1H and ^{31}P $\{^1H\}$ NMR spectra were recorded. Subsequently both samples were mixed and ^1H and $^{31}P(^1H)$ NMR spectra were recorded.

Identification of Products of Isomerizing Ethenolysis



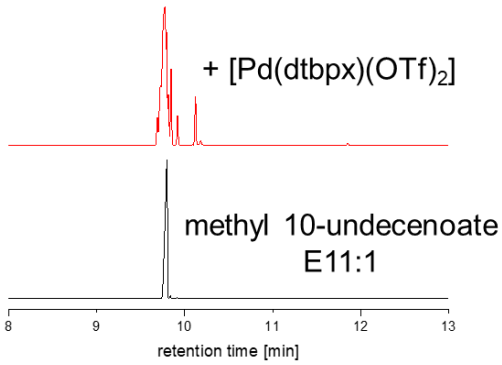


Figure 4-15: Gas chromatograms of commercially available genuine samples of different unsaturated esters (bottom, black) and the corresponding isomerized samples (top, red) (in MeOH after 1 h at 50°C).

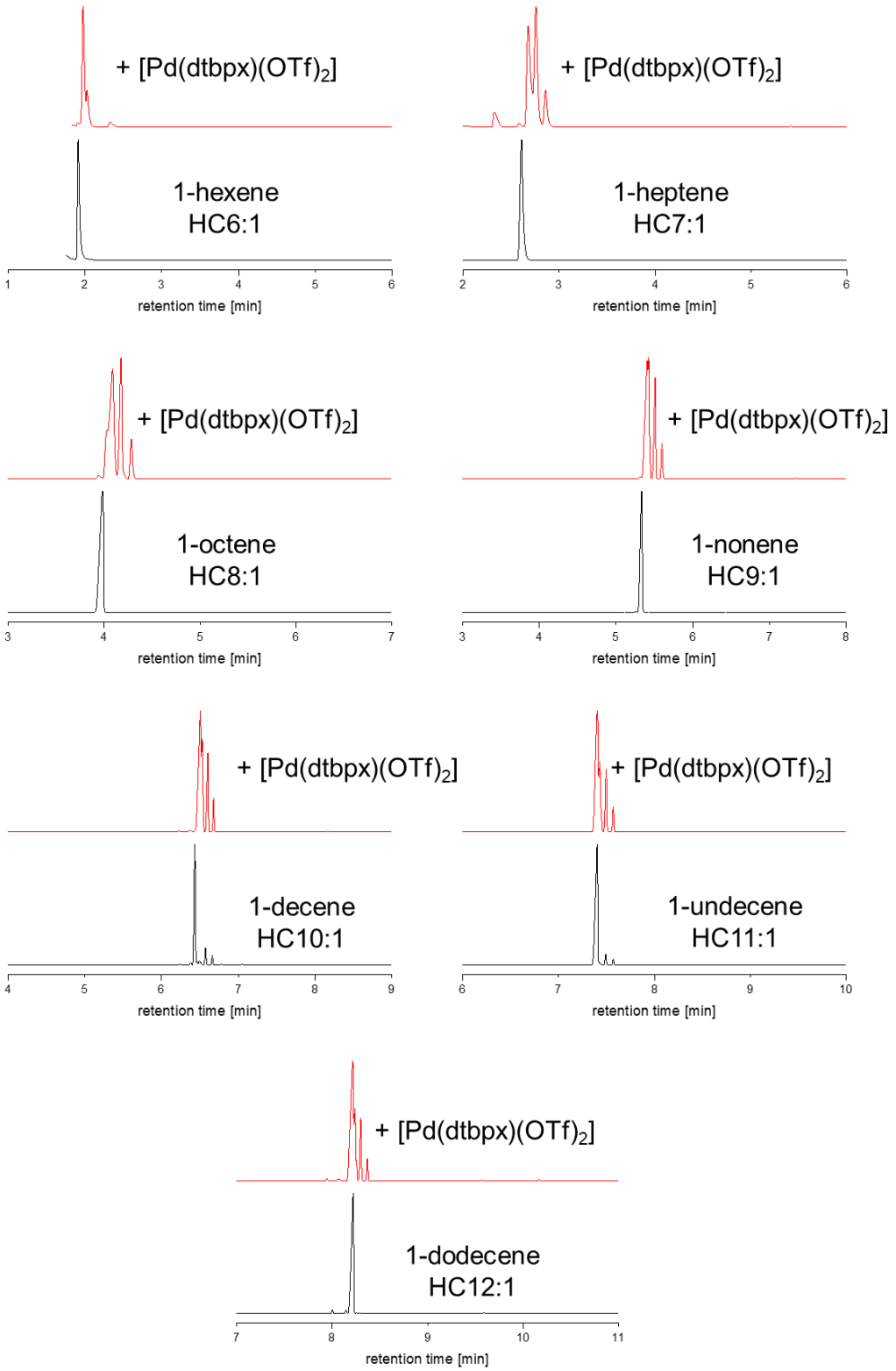


Figure 4-16: Gas chromatograms of commercially available genuine samples of different alkenes (bottom, black) and the corresponding isomerized samples (top, red) (in MeOH after 1 h at 50°C).

5 Integrated Extraction and Catalytic Upgrading of Microalgae Lipids in Supercritical Carbon Dioxide

5.1 Introduction

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 work-up is already complicated by the limited achievable cell densities. To address this problem, an integration of the biomass extraction with the catalytic upgrading in a common solvent, supercritical carbon dioxide ($scCO_2$) was explored (**Figure 5-1**).

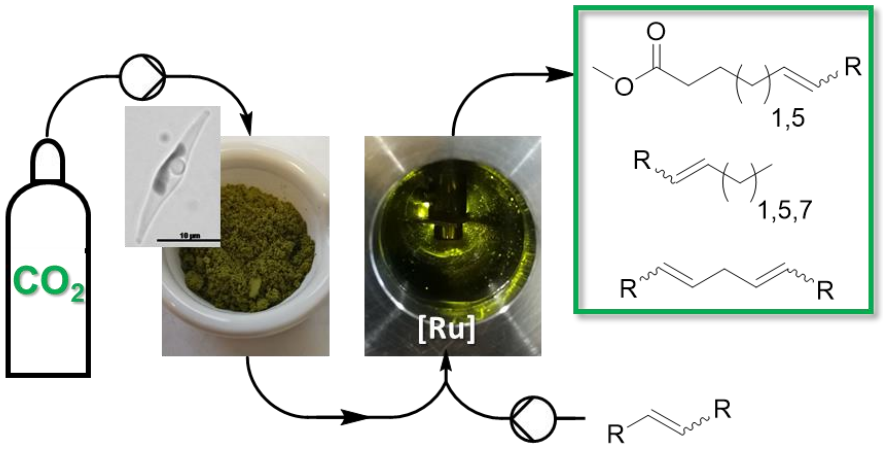


Figure 5-1: Schematic process design of extraction and catalytic valorization of microalgae in $scCO_2$ to mid-chain olefins and unsaturated esters.

Advantageously, $scCO_2$ is capable of a selective extraction of lipids from microalgae[95-98, 100, 101] and various catalytic reactions of interest have been reported to be compatible with $scCO_2$ as a solvent.[81, 91, 130-132] 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.[25] Considering microalgae oils as a feedstock, alkenolysis of their unconventional fatty acids (*vide supra*) can provide a range of

useful compounds, difficult to access otherwise.[133] Leitner and Fürstner have reported Ruthenium-based olefin metathesis to be compatible with $scCO_2$.[93, 108]

5.2 Results and Discussion

5.2.1 Extraction of Algae Oil in Supercritical Carbon Dioxide

The microalgae *Phaeodactylum tricornutum* was used for this studies, as this algae strain is robust and can contain high amounts of unsaturated fatty acids.[56] 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[134] (**Table 5-1**, **Figure 5-2**).

Table 5-1: Conditions and yields of $scCO_2$ extracted algae oil from freeze-dried algae of the strain *Phaeodactylum tricornutum*.

Entry	Pressure [bar]	Temperature [°C]	Density [g mL^{-1}][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^{-1} CO_2.[a]Density of neat carbon dioxide at the given temperatures and pressures were determined according to Ref 135. [b]Determined via GC with dodecane as an internal standard after transesterification with methanol.

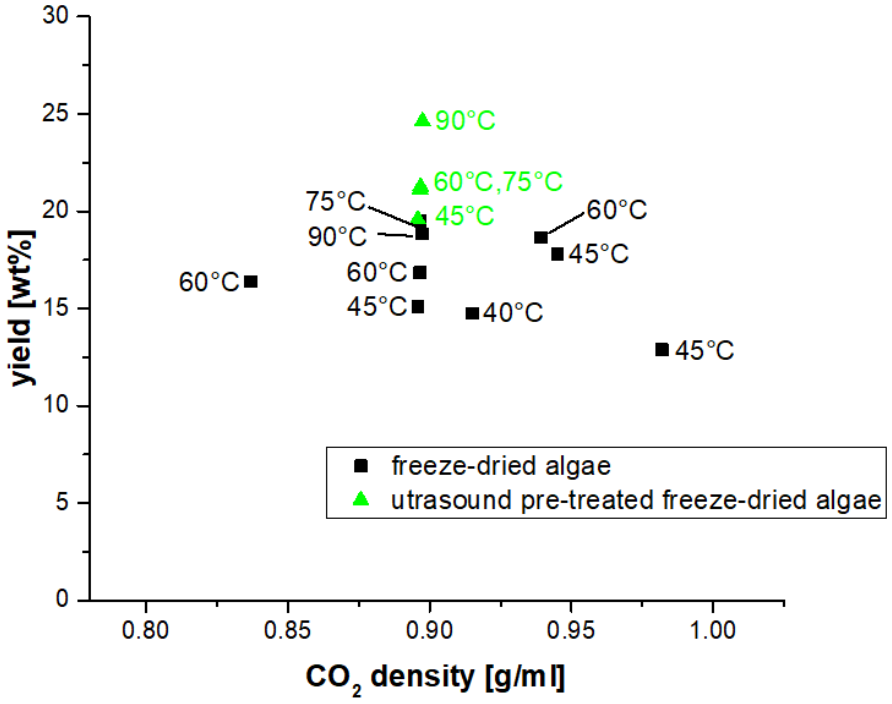


Figure 5-2: Yields of extraction of algae oil from *Phaeodactylum tricornutum* with $scCO_2$ at different densities. Corresponding pressures are given in Table 5-1.

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[106, 136] (**Figure 5-3**) 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 (**Figure 5-4**).

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.[69] This method, which quantitatively captures all fatty acid compounds present in the microalgae, yielded 28 wt% of extracted algae oil.

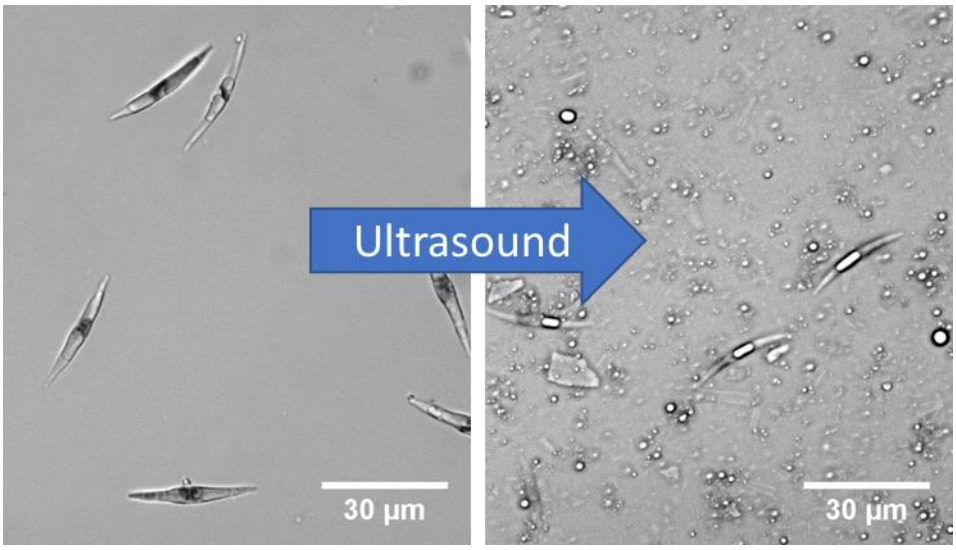


Figure 5-3: Light microscopy images of *Phaeodactylum tricornutum* before (left) and after cell disruption by ultrasound (right). The images were recorded on an Olympus BX 51 equipped with a Zeiss AxioCam MRm. Visible droplets can be attributed to intracellular and extracellular lipid droplets.

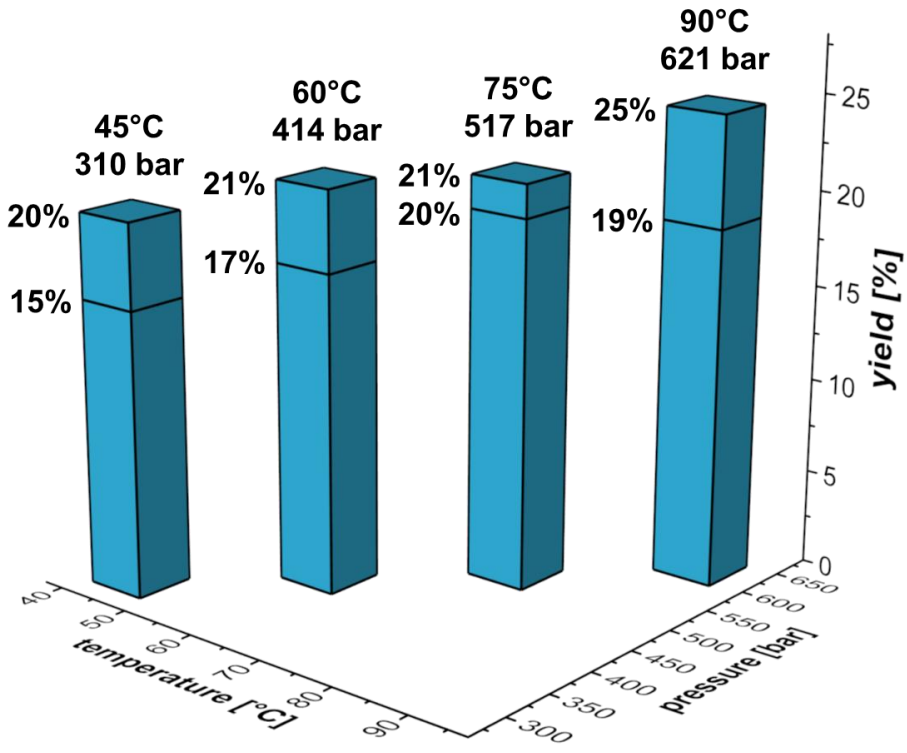


Figure 5-4: Yields of $scCO_2$ extraction at different pressures and temperatures at constant density (0.9 g mL^{-1}). Lower part of the bar: extraction of freeze-dried algae, lower and upper part of the bar: extraction of ultrasound pre-treated freeze-dried algae.

Gas chromatographic analysis of the extracted oils after transesterification with methanol reveals similar fatty acid compositions, largely independent of the $scCO_2$ extraction conditions (**Figure 5-5**). With only small variations (ca. 2%), 55% of mono-unsaturated fatty acid esters (47% methyl palmitoleate (FA16:1) and 8% methyl oleate (FA18:1)), 10% of the multiple unsaturated fatty acid ester methyl eicosapentaenoate (FA20:5), and 35% of saturated fatty acid esters (<1% stearic acid (FA18:0), 25% methyl palmitate (FA16:0) and 9% methyl myristate (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 (**Figure 3-2**).

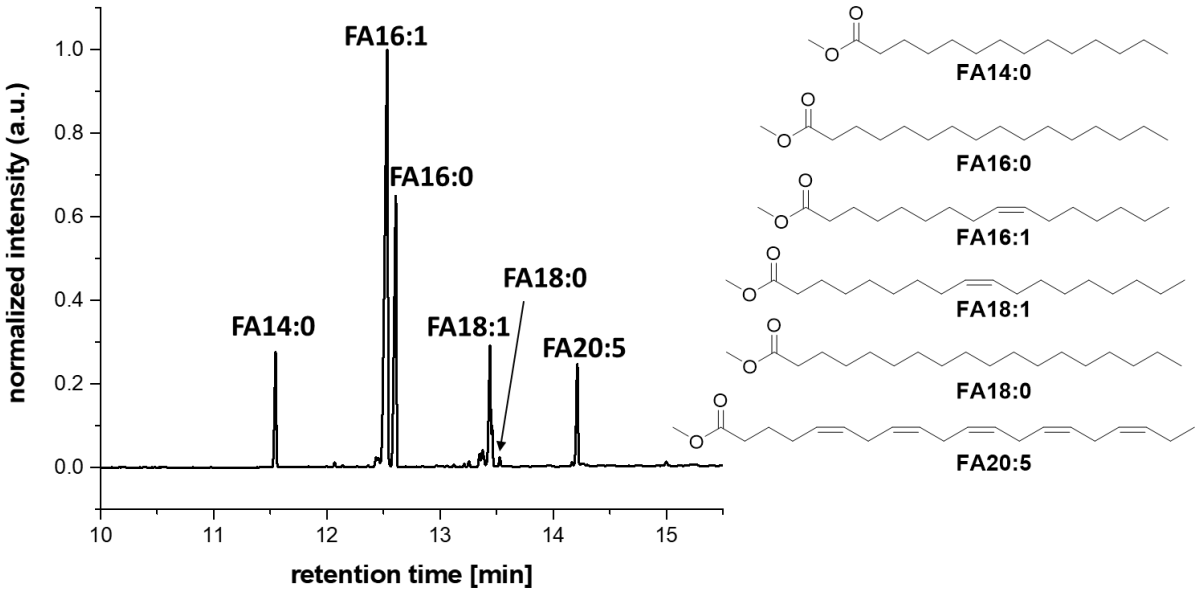


Figure 5-5: Gas chromatogram of $scCO_2$ extracted algae oil from *Phaeodactylum tricornutum* (after transesterification with methanol for GC analysis). Myristic acid methyl ester (FA14:0) 9%, palmitoleic acid methyl ester (FA16:1) 47%, palmitic acid methyl ester (FA16:0) 25%, oleic acid methyl ester (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 (**Table 5-2**), by Sandra Hess in her PhD thesis. The lipid composition was analyzed via thin-layer chromatography (TLC),[137, 138] pigments were quantified via high-performance liquid chromatography (HPLC)[139] and the content of proteins and carbohydrates was determined via Fourier-transform infrared spectroscopy (FT-IR)[140] Polar diacylglycerides[99] and chlorophylls[141] were found to not be extracted by the apolar $scCO_2$, in line with previous findings.[142] Proteins were also not detected in the investigated oils, 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 (**Figure 5-6**).

Figure 5-6: Appearance of algae oils extracted according to Folch (left) and with $scCO_2$ (right), respectively, diluted in CH_2Cl_2, showing the differences in pigment extraction. The Folch extraction also extracts chlorophylls, while $scCO_2$ extracts carotenoids selectively.

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 (**Figure 5-7**) as well as chlorophylls (**Figure 5-8**) are extracted from the microalgae samples (**Table 5-2**). This accounts for the differences in yields, that is, $scCO_2$ extraction under the conditions studied quantitatively extracts the triacylglycerides from the microalgae biomass.

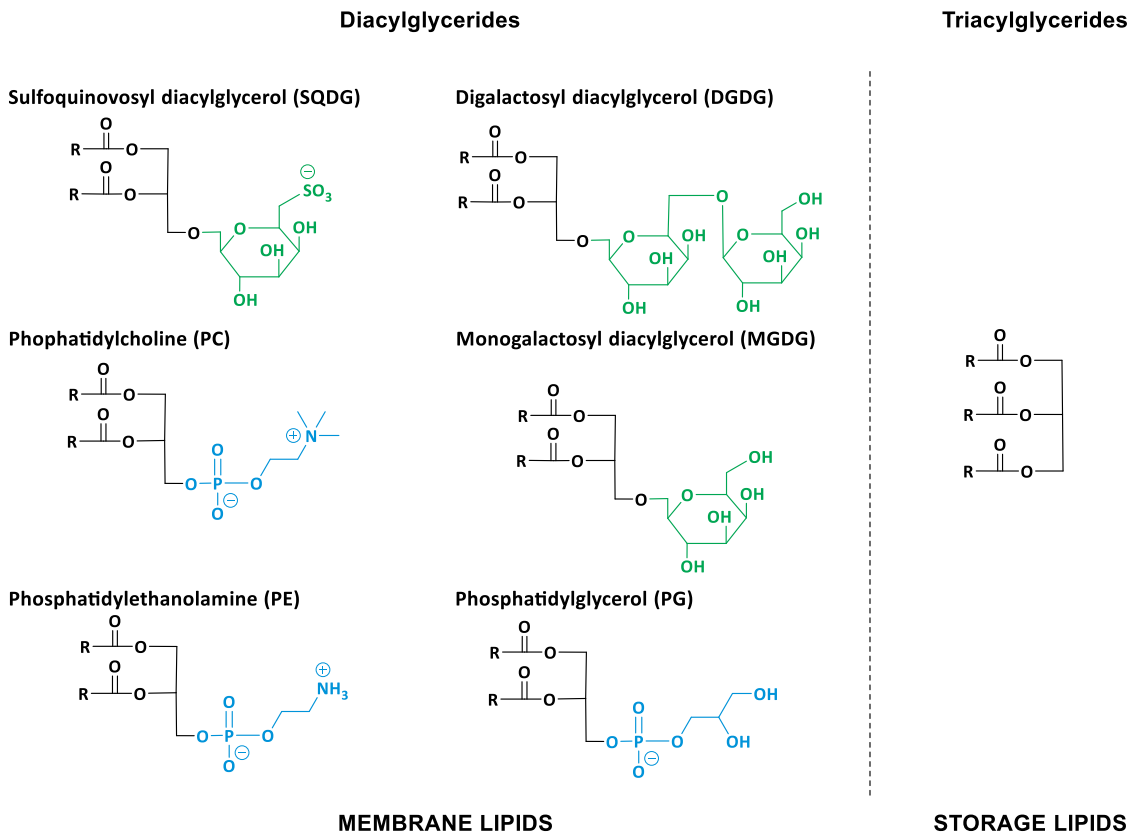


Figure 5-7: Lipid classes found in *Phaeodactylum tricornutum*. Carbohydrate moieties present in glycolipids are highlighted in green. Phosphorous/nitrogen containing moieties present in phospholipids are highlighted in blue. R = fatty acid residue.

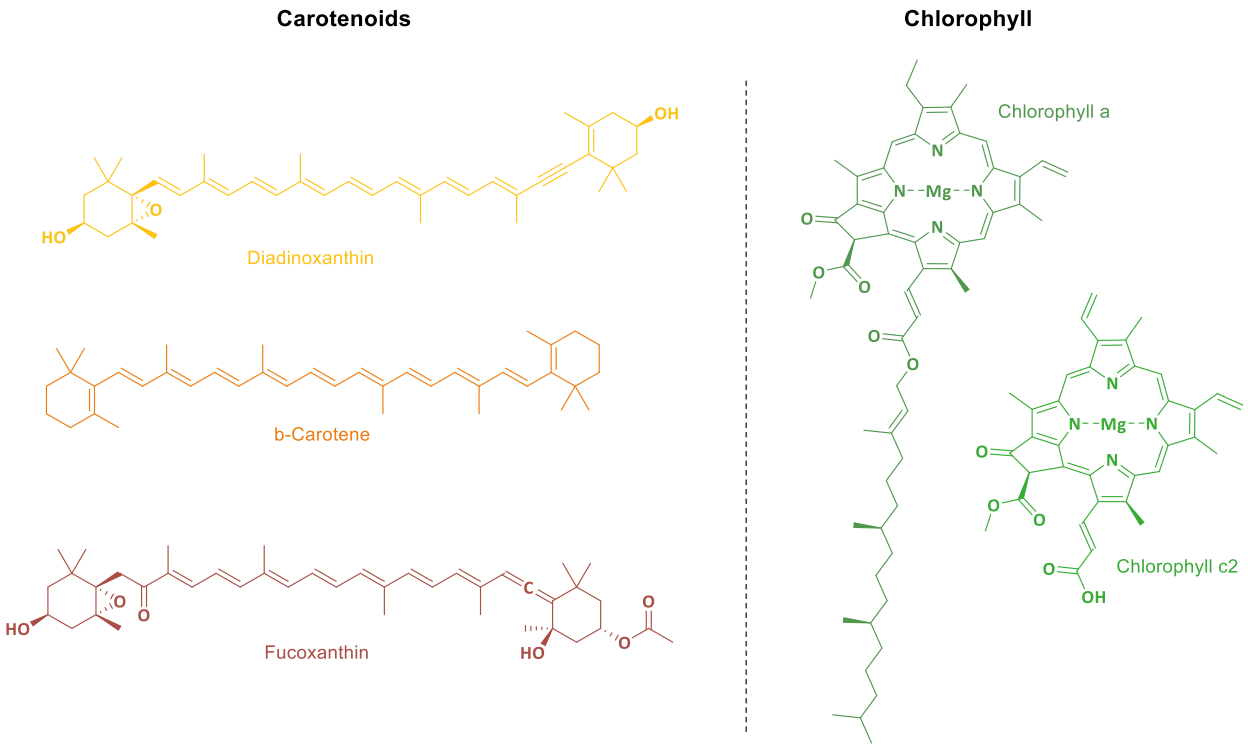


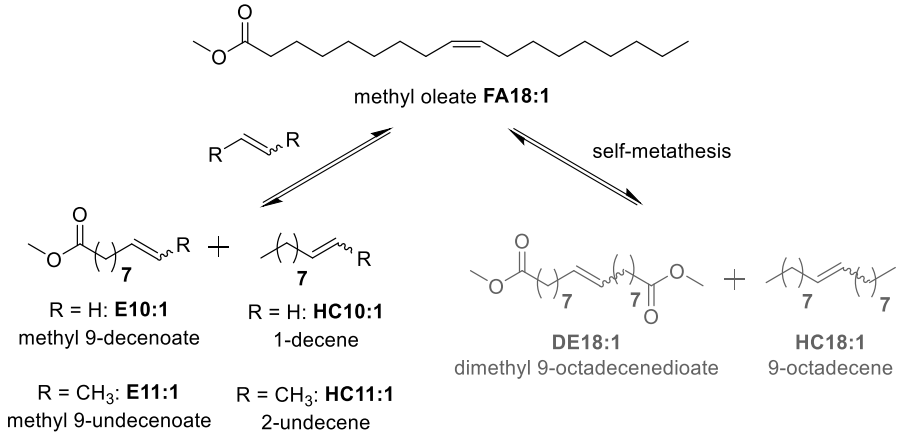
Figure 5-8: Pigments in *Phaeodactylum tricornutum*. The colors represent the color of the respective pigment.

Table 5-2: 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%

5.2.2 Ethenolysis in Supercritical Carbon Dioxide

Catalytic upgrading of fatty acids from microalgae via cross-metathesis gives access to a broad spectrum of unsaturated compounds. A general scheme of cross-metathesis of methyl oleate with ethylene or 2-butene is shown in **Scheme 5-1**.



Scheme 5-1: Self- and cross-metathesis of FA18:1 with ethylene (R=H) or 2-butene ($R=CH_3$).

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 5-1**, 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.*[115], 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 5-3**). Dimethyl 9-octadecendioate (DE18:1) and 9-octadecene (HC18:1), the self-metathesis products of methyl oleate (**Scheme 5-1**), were not observed (<1%). Due to promising results with Hoveyda-Grubbs 1st generation catalyst in the ethenolysis of methyl oleate in dichloromethane (c.f. chapter 4.2.1, **Table 4-1**), 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), ethenolysis applying Hoveyda-Grubbs 1st generation catalyst gave a higher conversion of 88%. 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 (**Table 5-3**).

Table 5-3: 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,[135] plus amount of ethylene in gas phase). [a]Determined via GC analysis. [b]The remainder are self-metathesis products.

Compared to ethenolysis of methyl oleate as model compound in dichloromethane (c.f. chapter 4.2.1, **Table 4-1**) at a catalyst loading of 0.5 mol% and an ethylene pressure of 10 bar, the selectivity is similar in both solvents, whereas the conversion in dichloromethane is with 95% slightly higher.

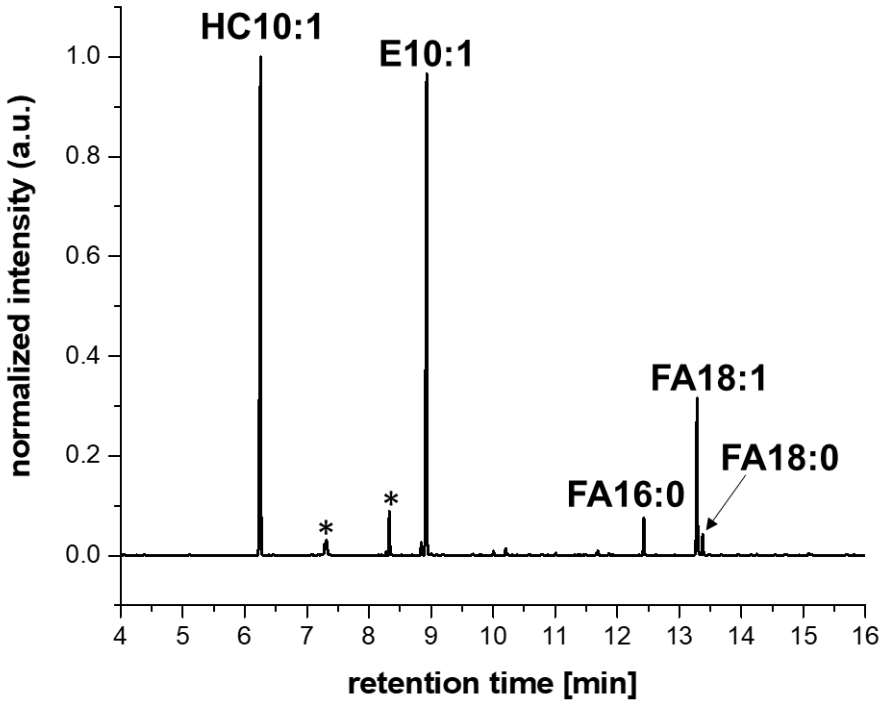


Figure 5-9: Gas chromatogram of the reaction mixture of ethenolysis in $scCO_2$ of methyl oleate with 0.5mol% Hoveyda-Grubbs 1st generation catalyst. The starting material (methyl oleate (FA18:1)) and the ethenolysis products (methyl 9-decenoate (E10:1), 1-decene (HC10:1)) are assigned. Side-products likely originating from ethyl vinyl ether quenching are marked with an asterisk.

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 corresponding to 0.75 g mL^{-1}).[108] 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 (**Table 5-3** and chapter 4.2.1, **Table 4-1**) 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: 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). Note that, 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 favours 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 5-4**, column "model substrate mixture").

Table 5-4: Ethenolysis 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, c.f. Table 5-8 and Table 5-9 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.

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 complete self-metathesis of FA20:5 (formation of two equivalents CHD).

Like in 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 5-4**). 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 6h.

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.

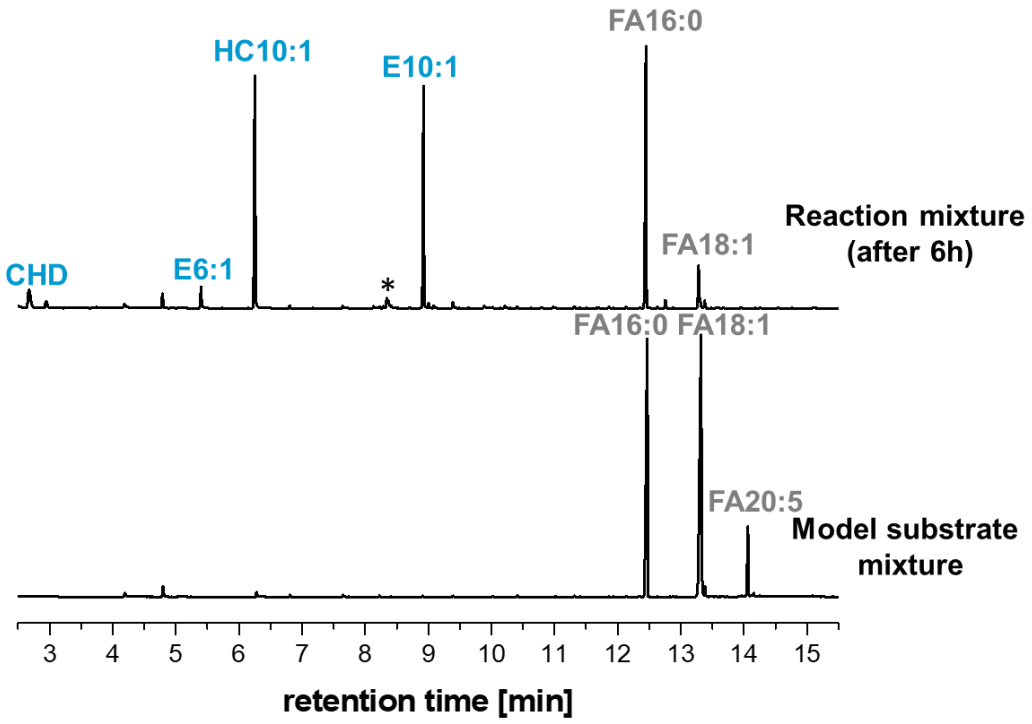


Figure 5-10: Gas chromatograms of the model substrate mixture (bottom) and the reaction mixture of the ethenolysis in $scCO_2$ of this model substrate mixture (top) with Hoveyda-Grubbs 1st generation catalyst and assignments of the products (1,4-cyclohexadiene (CHD), methyl 5-hexenoate (E6:1), 1-decene (HC10:1), methyl 9-decenoate (E10:1)) and the fatty acid esters (methyl palmitate (FA16:0), methyl oleate (FA18:1), methyl eicosapentaenoate (FA20:5)). Side-products likely originating from ethyl vinyl ether quenching are marked with an asterisk.

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 after transesterification with methanol (**Figure 5-11**) 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 (**Table 5-4**). Also, the conversion of the five-fold unsaturated fatty acid 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.

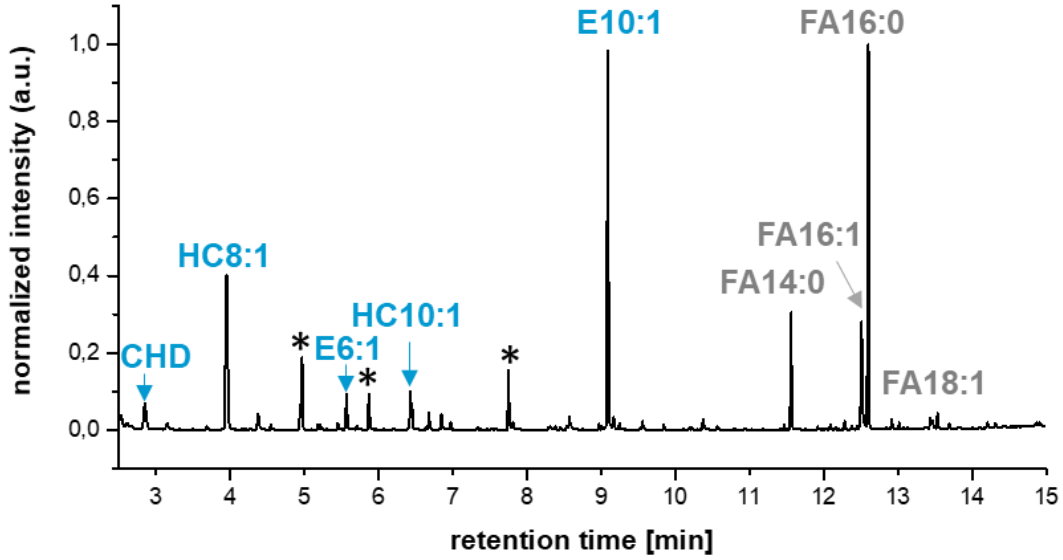


Figure 5-11: Gas chromatogram of the ethenolysis of $scCO_2$ extracted algae oil in $scCO_2$ (after transesterification with methanol) applying Hoveyda-Grubbs 1st generation catalyst and assignments of the ethenolysis products (1-octene (HC8:1), methyl 5-hexenoate (E6:1), 1-decene (HC10:1), methyl 9-decenoate (E10:1)), the self-metathesis product 1,4-cyclohexadiene (CHD) and the fatty acid esters (methyl myristate (FA14:0), methyl palmitoleate (FA16:1), methyl palmitate (FA16:0), methyl oleate (FA18:1)). Side-products likely originating from ethyl vinyl ether quenching are marked with an asterisk.

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. All these results in general match with butenolysis of methyl oleate in dichloromethane as solvent (c.f. chapter 3.2.2).

5.2.3 Butenolysis in Supercritical Carbon Dioxide

Besides ethenolysis, the cross-metathesis with internal alkenes such as 2-butene (c.f. chapter 3) 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.[22-24]

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 this catalyst showed a high selectivity and conversion in butenolysis in dichloromethane as demonstrated in chapter 3. 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) (c.f. **Scheme 5-1**, R = Me). Conditions similar to butenolysis in dichloromethane were adopted (10 equivalents of 2-butene and 0.1 mol% Hoveyda-Grubbs 2nd generation catalyst),[133] 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 (**Figure 5-12**). 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 (c.f. chapter 3.2.2).[30, 133] 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 5-5**).

Table 5-5: 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 catalyst, 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.

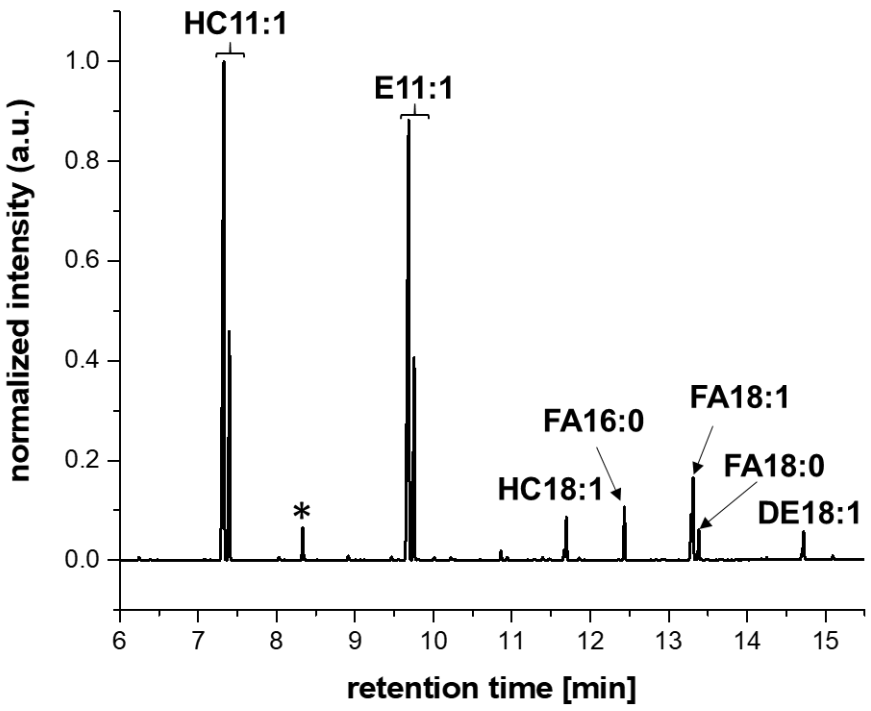


Figure 5-12: Gas chromatogram of the reaction mixture of butenolysis in $scCO_2$ of methyl oleate with 0.1 mol% Hoveyda-Grubbs 2nd generation catalyst. The starting material (methyl oleate (FA18:1)), the butenolysis products (methyl 9-undecenoate (E11:1), 2-undecene (HC11:1), both *trans* and *cis* isomers in a ratio of 80:20) and self-metathesis products (9-octadecene (HC18:1), dimethyl 9-octadecenedioate (DE18:1)) are assigned. Side-products likely originating from ethyl vinyl ether quenching are marked with an asterisk.

Compared to ethenolysis of methyl oleate in $scCO_2$, the catalyst loading required for maximum conversion in butenolysis (0.1 mol%) is fivefold lower. As previously stated, the formation of the unstable methylidene intermediate catalyst 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 in order to elucidate the butenolysis products of algae oil. The following butenolysis products were expected: 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.

Table 5-6: Butenolysis 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, c.f. Table 5-11 and Table 5-12 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).

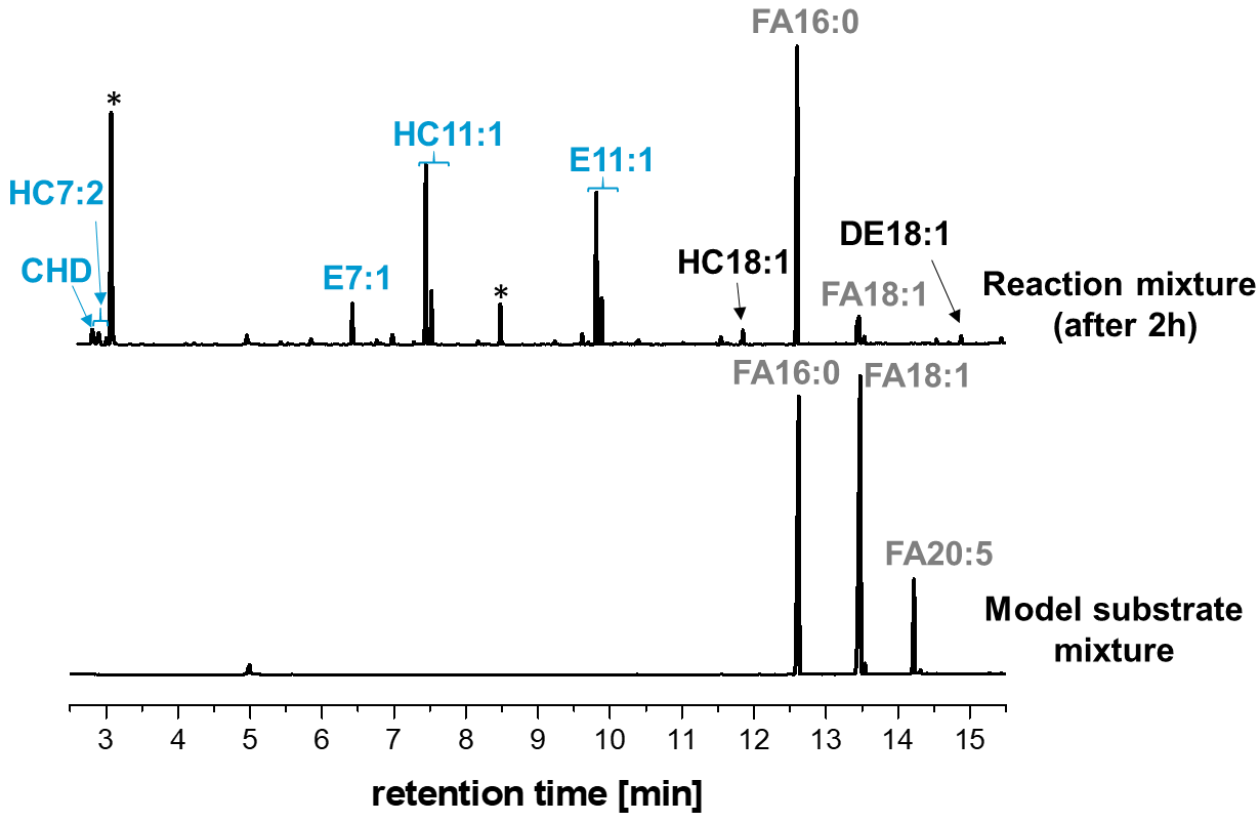


Figure 5-13: Gas chromatograms of a model substrate mixture (bottom) and the reaction mixture of the butenolysis in $scCO_2$ of this model substrate mixture (top) with Hoveyda-Grubbs 2nd generation catalyst and assignments of the butenolysis products (2,5-heptadiene (HC7:2), methyl 5-heptenoate (E7:1), 2-undecene (HC11:1), methyl 9-undecenoate (E11:1)), self-metathesis products (1,4-cyclohexadiene (CHD), 9-octadecene (HC18:1), dimethyl 9-octadecenedioate (DE18:1)) and the fatty acid esters (methyl palmitate (FA16:0), methyl oleate (FA18:1), methyl eicosapentaenoate (FA20:5)). Side-products likely originating from ethyl vinyl ether are marked with an asterisk.

Based on these results, butenolysis of $scCO_2$ extracted algae oil (**Table 5-6**, column "$scCO_2$ extracted algae oil") 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 (**Figure 5-14**). With 91% and 99% the conversions of FA18:1 and the five-fold unsaturated fatty, respectively, agree with the conversions observed for the mixture of model compounds (**Table 5-6**). 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.

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 in

$scCO_2$. Catalyst performance regarding selectivity and conversion is also comparable to the results obtained in the ethenolysis of algae oil in dichloromethane as solvent (c.f. chapter 3.2.2).

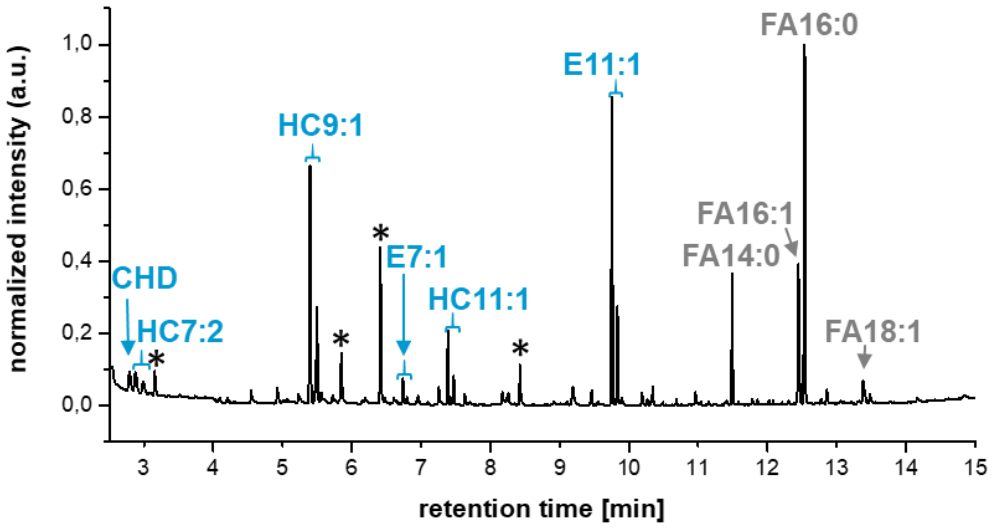


Figure 5-14: Gas chromatogram of butenolysis in $scCO_2$ of $scCO_2$ extracted algae oil (after transesterification with methanol) with Hoveyda-Grubbs 2nd generation catalyst and assignments of the butenolysis products (2,5-heptadiene (HC7:2), 2-nonene (HC9:1), methyl 5-heptenoate (E7:1), 2-undecene (HC11:1), methyl 9-undecenoate (E11:1)) and the fatty acid esters (methyl myristate (FA14:0), methyl palmitoleate (FA16:1), methyl palmitate (FA16:0), methyl oleate (FA18:1)). Side-products likely originating from ethyl vinyl ether are marked with an asterisk.

5.2.4 Simultaneous Extraction and Cross-Metathesis in $scCO_2$

As demonstrated in the previous sections, $scCO_2$ 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, a combined approach of extraction and cross-metathesis of microalgae in $scCO_2$ is proposed.

The combination of extraction and ethenolysis or butenolysis was performed at a $scCO_2$ pressure of 300 bar at 45 °C. For each combination of extraction and metathesis reaction, 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.

Table 5-7: 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, c.f. Table 5-10 and Table 5-13 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 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 internal standard) in GC. [e]Not detectable via GC due to its low boiling point.

Subjecting the freeze-dried algae to the combined extraction and ethenolysis results in a conversion of 32% and 44% (**Table 5-7**) for FA16:1 and FA18:1, respectively. In contrast, the conversion of 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 is almost identical (88% for combined and 92% for two-step approach), the mono-unsaturated fatty acids 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 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.

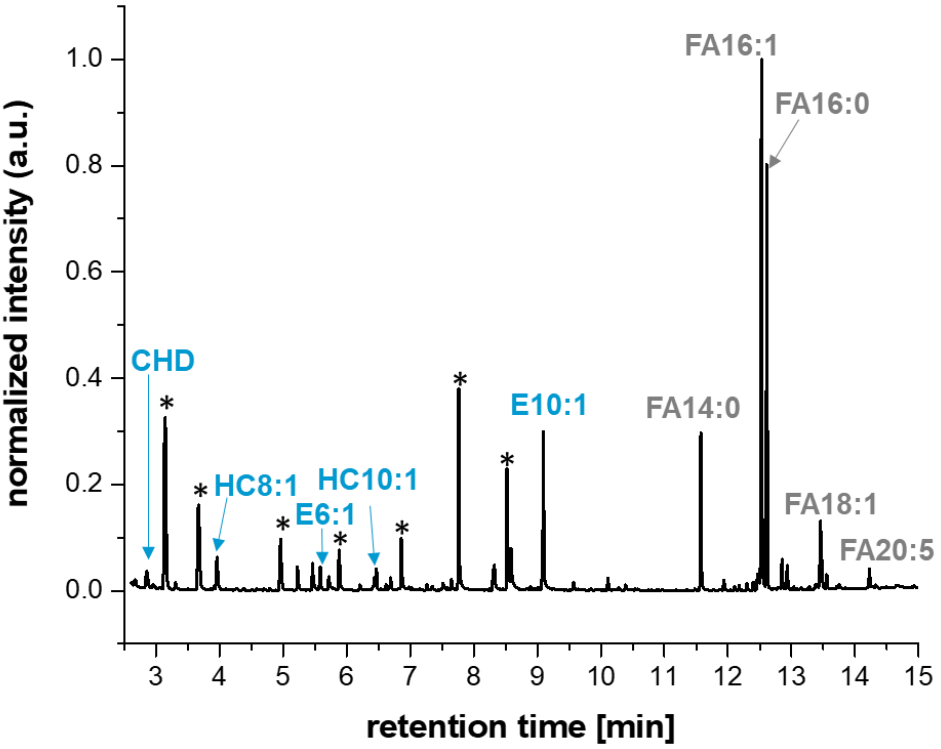


Figure 5-15: Gas chromatogram (after transesterification with methanol) of ethenolysis in $scCO_2$ of ultrasound pre-treated freeze-dried algae with Hoveyda-Grubbs 1st generation catalyst and assignments of the ethenolysis products (1-octene (HC8:1), methyl 5-hexenoate (E6:1), 1-decene (HC10:1), methyl 9-decenoate (E10:1)), the self-metathesis product 1,4-cyclohexadiene (CHD) and the fatty acid esters (methyl myristate (FA14:0), methyl palmitoleate (FA16:1), methyl palmitate (FA16:0), methyl oleate (FA18:1), methyl eicosapentaenoate (FA20:5)). Side-products likely originating from ethyl vinyl ether quenching are marked with an asterisk.

In the combined extraction and butenolysis of pre-treated freeze-dried algae (**Table 5-7**) 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 of 89% is still in the same range as for the butenolysis two-step process.

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

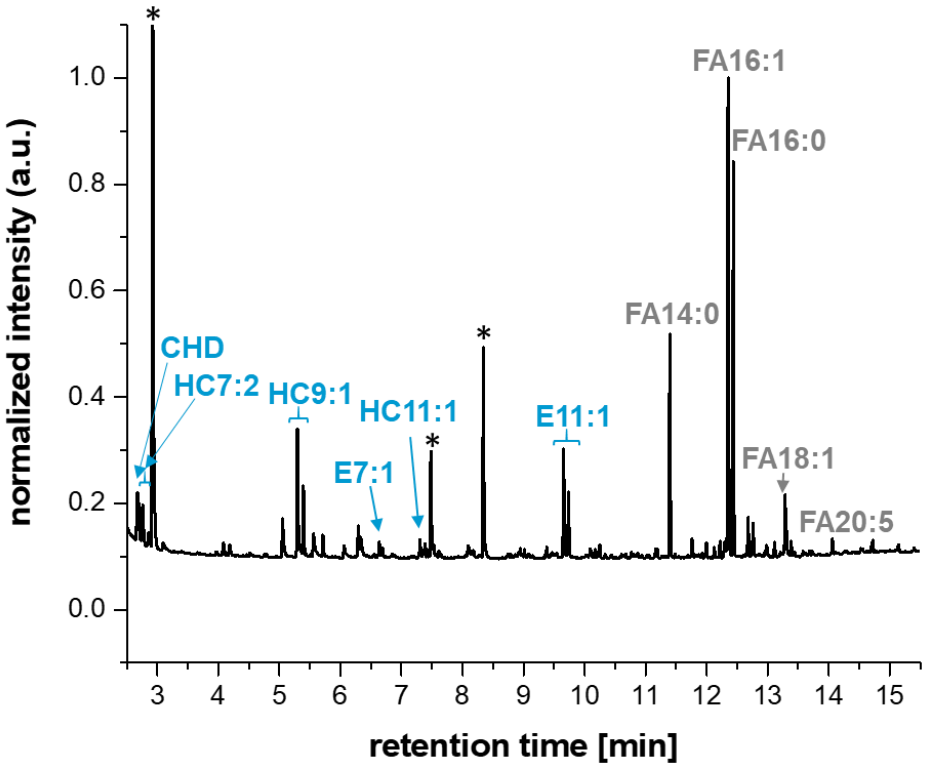


Figure 5-16: Gas chromatogram (after transesterification with methanol) of butenolysis in $scCO_2$ of ultrasound pre-treated freeze-dried algae with Hoveyda-Grubbs 2nd generation catalyst and assignments of the butenolysis products (2,5-heptadiene (HC7:2), 2-nonene (HC9:1), methyl 5-heptenoate (E7:1), 2-undecene (HC11:1), methyl 9-undecenoate (E11:1)), the self-metathesis product 1,4-cyclohexadiene (CHD) and the fatty acid esters (methyl myristate (FA14:0), methyl palmitoleate (FA16:1), methyl palmitate (FA16:0), methyl oleate (FA18:1)). Side-products likely originating from ethyl vinyl ether quenching are marked with an asterisk.

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.

5.3 Conclusion

Fatty acids from microalgae are attractive compounds for catalytic upgrading to chemicals, but their extraction often requires energy-intensive multi-step procedures and the use of various organic solvents. To relieve this bottleneck, a straightforward approach of combined extraction and catalytic functionalization via olefin cross-metathesis (ethenolysis and butenolysis) in supercritical CO_2 ($scCO_2$) was demonstrated. At optimum conditions (90 °C, 620 bar, $\rho(CO_2) = 0.90$ g mL^{-1}) the desired lipids from the microalgae strain *Phaeodactylum tricornutum* were extracted selectively and quantitatively from previously disrupted cells, while organic solvent extraction for comparison additionally extracted polar diacylglycerides and chlorophylls.

The unsaturated fatty acid esters (FA16:1, FA18:1 and FA20:5) were converted in a one-pot approach by catalytic olefin ethenolysis or butenolysis, respectively, to target mid-chain olefins and unsaturated esters (**Figure 5-17**). The product spectrum compares to alkenolysis of individual model compounds in $scCO_2$ as well as of separately $scCO_2$ extracted microalgae oil. 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 for the case of olefin metathesis, can help to overcome the bottleneck biomass extraction represents for its utilization as a feedstock, and of microalgae in particular.

Figure 5-17: Schematic representation of the integrated extraction and catalytic upgrading of algae oil to higher value chemical building blocks.

5.4 Experimental Section

5.4.1 General Considerations

All solvents were standard analytical grade and used as received. 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%). Methyl eicosapentaenoate and Hoveyda-Grubbs 2nd generation catalyst were purchased from Carbosynth. Methyl eicosapentaenoate was distilled in vacuum prior to use. Methyl palmitate was purchased from Nu-Check Prep. *Cis/trans*-2-butene (99%) 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.

After harvest, wet and dried microalgal cells and all extracts were protected from light to prevent oxidation. 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. The oven was kept at 50 °C for 3 min, then heated with 20 °C min^{-1} to 280 °C, and kept isothermal at 280 °C for 5 min. The split was defined as a 20 ml min^{-1} inlet flow and 1.5 ml min^{-1} column flow. All free acids were esterified with methanol prior to GC analysis. The fatty acid esters and metathesis products were identified via comparison of retention times and enrichment experiments with commercially available genuine standards.

5.4.2 High-Pressure Instrumentation

The high-pressure reactor (**Figure 5-18**) 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 steel 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).

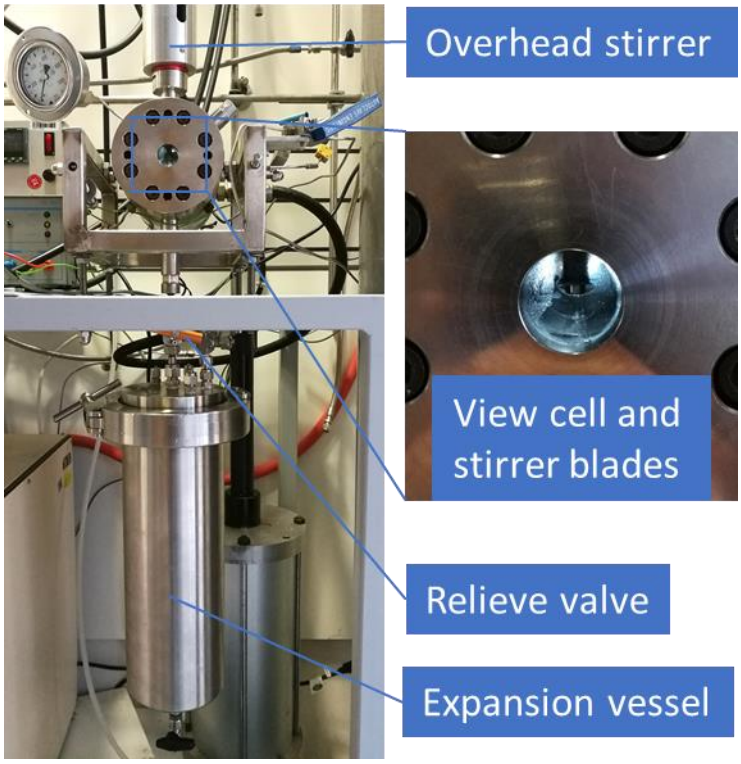


Figure 5-18: High-pressure reactor employed.

A supercritical fluid extractor SFX-110W (**Figure 5-19**) 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.

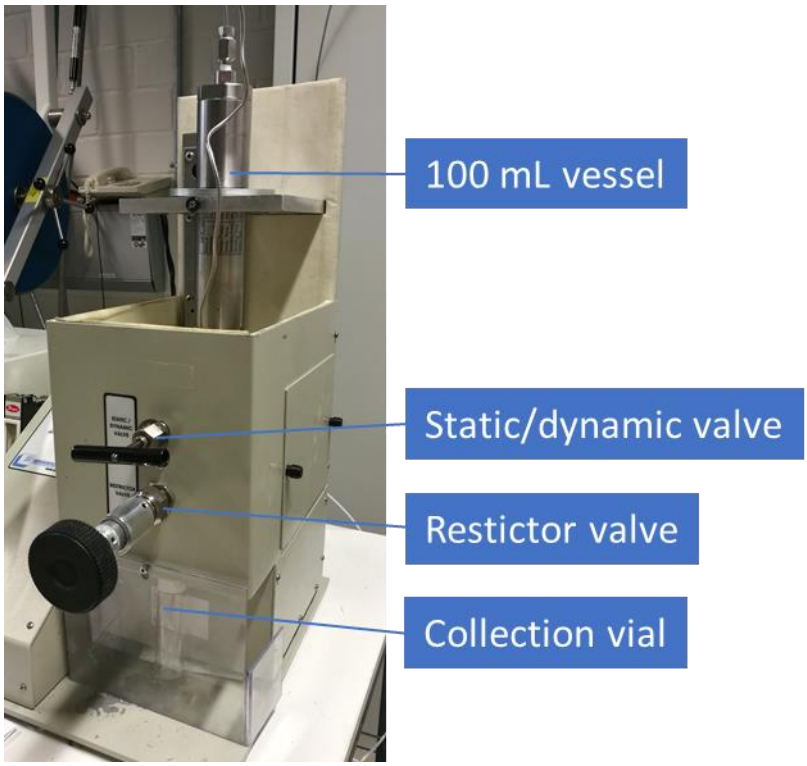


Figure 5-19: Supercritical fluid extractor SFX-110W.

5.4.3 Algae Cultivation and Harvest

The examined strain is a single clone colony of *Phaeodactylum tricornutum* (*P. tricornutum*) wildtype UTEX 646 (WT4). Algae were grown in 10 L flasks in a f2 medium[127] (pH=7) with artificial half concentrated sea salts (16.6 g L^{-1}, tropic marine) and at 20 °C in continuous light with a light intensity of 35 µMol s^{-1} m^{-2}. The cultures were aerated with ambient air during the six weeks cultivation period.

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 Heraeus (Hanau, Germany). The second centrifugation step was conducted with an Allegra 25R centrifuge (5000 g, 10 min at 4 °C) from Beckman coulter. The crude wet algae were further freeze-dried to obtain crude dried algae. Optionally, the wet algae were pre-treated by ultrasonication 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.

5.4.4 Extraction Methods

Organic Solvent Extraction

Organic solvent extraction was performed according to Folch *et al*.[69] 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 was dried in vacuum. The obtained algae oil was stored at -20 °C.

Supercritical CO_2 Extraction

In a 5-micron mesh nylon sample bag, 1 g freeze dried algae or ultrasound pre-treated freeze-dried algae, respectively, were 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 5-19**), pressurized and heated. For optimization of $scCO_2$ extraction different pressures were applied. For characterization, the algae oil was extracted at 414 bar. The algae were allowed to soak for 20 minutes. Subsequently, the algae oil was extracted employing a 10 mL min^{-1} 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.

5.4.5 Ethenolysis in $scCO_2$

General Procedure for Ethenolysis in $scCO_2$

All experiments were carried out in the $scCO_2$ pressure reactor (**Figure 5-18**) 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.

Ethenolysis of a Model Substrate Mixture in $scCO_2$

A ratio of 4:5:1 (methyl palmitate FA16:0 : methyl oleate FA18:1 : methyl eicosapentaenoate 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.

Table 5-8: Ethenolysis of unsaturated fatty acids of a model substrate mixture with conversions of the components and selectivities to ethenolysis products and self-metathesis product 1,4-cyclohexadiene (CHD).

		1	**2**
Composition of the initial reaction mixture [%]	FA16:0	40	40
	FA18:1	50	50
	FA20:5	10	10
Conversion [%][a]	FA18:1	85	86
	FA20:5	>99	>99
Selectivity [%][b] for	CHD	82	86
	E6:1	83	87
	HC10:1	>99	>99
	E10:1	>99	>99

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]Determined over FA16:0 as an internal standard. [b]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.

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. All products were identified via comparison of retention times with commercially available genuine standards (**Figure 5-20**).

Table 5-9: Ethenolysis of unsaturated fatty acids of $scCO_2$ extracted algae oil with conversions of the components and selectivities to ethenolysis products and self-metathesis product 1,4-cyclohexadiene (CHD).

		1	**2**
Composition of the initial reaction mixture [%]	FA14:0	9	9
	FA16:1	49	49
	FA16:0	27	27
	FA18:1	4	4
	FA18:0	2	2
	FA20:5	9	9
Conversion [%][a]	FA16:1	83	79
	FA18:1	88	92
	FA20:5	94	89
Selectivity [%][b] for	CHD	61	68
	E6:1	84	79
	HC8:1	79	86
	HC10:1	>99	94
	E10:1	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]Determined over FA16:0 as an internal standard. [b]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.

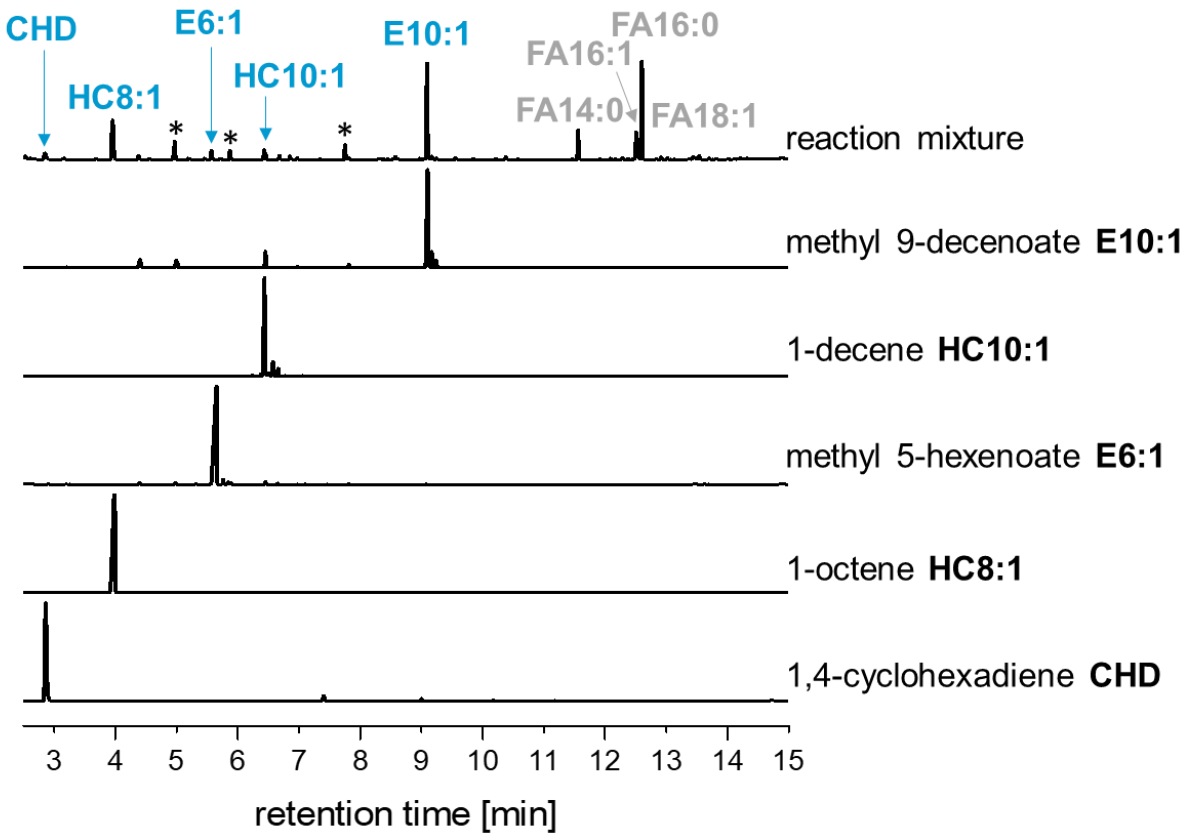


Figure 5-20: Gas chromatogram of the ethenolysis of $scCO_2$ extracted algae oil in $scCO_2$ (after transesterification with methanol) with Hoveyda-Grubbs 1st generation catalyst (top) and gas chromatograms of genuine samples of the ethenolysis products and the self-metathesis product 1,4-cyclohexadiene CHD for comparison. Side-products likely originating from ethyl vinyl ether quenching are marked with an asterisk.

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 catalyst 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 to 45 °C. When the temperature was reached, 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.

Table 5-10: Integrated procedure of extraction and ethenolysis of freeze-dried algae in $scCO_2$ with selectivities and conversions of the unsaturated fatty acids.

		1	**2**
Conversion [%][a]	FA16:1	31	34
	FA18:1	46	41
	FA20:5	86	90
Selectivity [%][b] for	CHD	46	53
	E6:1	92	83
	HC8:1	63	56
	HC10:1	88	88
	E10:1	78	76

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. Conditions: 0.5 mol% Hoveyda-Grubbs 1st generation catalyst, 10 bar ethylene, 300 bar CO_2 (total pressure) at 45°C, 18 h. [a]Conversions were determined via gas chromatography via FA16:0 present in the algae oil as an internal standard. [b]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 in case of CHD (1,4-cyclohexadiene) complete self-metathesis of FA20:5 (determined over FA16:0 as an internal standard) in GC.

5.4.6 Butenolysis in $scCO_2$

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 to 45 °C. When the temperature was reached, 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.

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 catalyst) were used. The experiments were carried out at a constant reactor volume of 30 mL.

Table 5-11: Butenolysis of unsaturated fatty acids of a model substrate mixture with conversions of the components and selectivities for butenolysis products and self-metathesis product 1,4-cyclohexadiene (CHD).

		1	**2**
Composition of the initial reaction mixture [%]	FA16:0	40	40
	FA18:1	50	50
	FA20:5	10	10
Conversion [%][a]	FA18:1	88	88
	FA20:5	>99	95
Selectivity [%][b] for	CHD	25	29
	HC7:2	55	49
	E7:1	65	68
	HC11:1	88	81
	E11:1	88	87

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]Determined via FA16:0 as an internal standard. [b]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.

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 catalyst) 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.

Table 5-12: Butenolysis of unsaturated fatty acids of $scCO_2$ extracted algae oil with conversions of the components and selectivities for butenolysis products and self-metathesis product 1,4-cyclohexadiene (CHD).

		1	**2**
Composition of the initial reaction mixture [%]	FA14:0	9	9
	FA16:1	47	47
	FA16:0	25	25
	FA18:1	8	8
	FA18:0	1	1
	FA20:5	10	10
Conversion [%][a]	FA16:1	79	83
	FA18:1	93	88
	FA20:5	>99	>99
Selectivity [%][b] for	CHD	26	22
	HC7:2	57	54
	E7:1	67	63
	HC9:1	88	82
	HC11:1	84	82
	E11:1	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]Determined via FA16:0 as an internal standard. [b]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.

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 catalyst 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 to 45 °C. When the temperature was reached, 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.

Table 5-13: Integrated procedure of extraction and butenolysis of freeze-dried algae in $scCO_2$ with selectivities and conversions of the unsaturated fatty acids.

		1	**2**
Conversion [%][a]	FA16:1	44	50
	FA18:1	65	64
	FA20:5	91	87
Selectivity [%][b] for	CHD	34	37
	HC7:2	67	72
	E7:1	64	71
	HC9:1	91	>99
	HC11:1	>99	95
	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. 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. [a]Conversions were determined via gas chromatography via FA16:0 present in the algae oil as an internal standard. [b]The selectivity for 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 over FA16:0 as an internal standard) in GC.

5.4.7 Further Gas Chromatographic Data

Additional gas chromatographic signals from quenching with ethyl vinyl ether

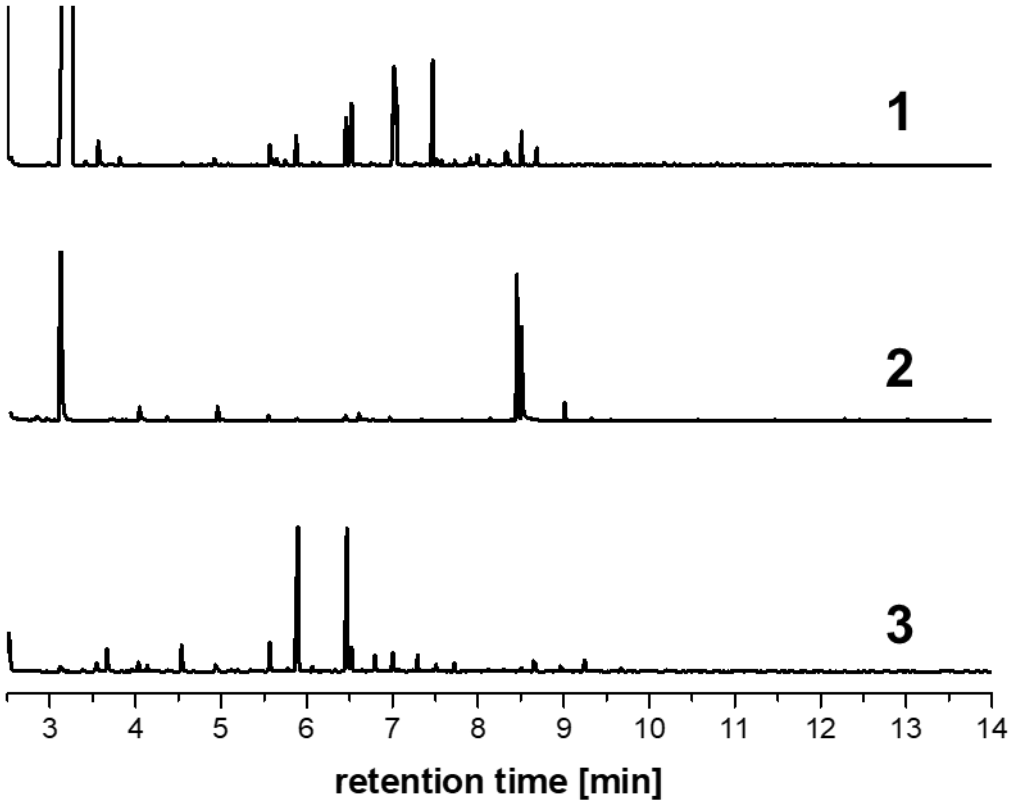


Figure 5-21: 1: Gas chromatogram of Hoveyda-Grubbs 1st generation catalyst in methanol, dichloromethane and a catalytic amount of sulfuric acid quenched with ethyl vinyl ether. 2: Gas chromatogram of Hoveyda-Grubbs 2nd generation catalyst in methanol, dichloromethane and a catalytic amount of sulfuric 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.

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6 Conclusive Summary

Microalgae are an attractive alternative lipid source compared to traditional oil plants such as sunflower, rapeseed or soybean. They do not compete with food production as microalgae can be grown in salt or brackish water and no arable land is required. One further advantage is their high growth rate, some microalgae strains can double their biomass within 24 h. Furthermore, microalgae oil has a special composition: It contains fatty acids with unusual chain length and high amounts of (poly-)unsaturated fatty acids such as eicosapentaenoic acid (FA20:5) and docosahexaenoic acid (FA22:6) both barley found in traditional plant oils. These ω-3 fatty acids are employed increasingly in the nutrition and health industry. The utilization of microalgae oil for the production of biofuel has also been studied extensively. However, this application does not exploit the full potential of this special feedstock and the unique structure of fatty acids in general. The long hydrocarbon chain, the carboxyl group and in case of (poly-)unsaturated fatty acids the double bond(s) make them attractive for catalytic functionalization to higher value chemicals.

In this work the unicellular marine diatom *Phaeodactylum tricornutum* was chosen as source of lipids as this algae strain is robust and contains high amounts of unsaturated fatty acids. Organic solvent lipid extraction via a modified Folch method from a 30 L algae culture in the stationary phase typically yielded 6-7 g crude oil (**Figure 6-1**).

This microalgae oil serves as a feedstock for a biorefinery approach including butenolysis and isomerizing alkoxycarbonylation to obtain mid-chain (di-)carboxylic acid esters, otherwise only accessible via demanding synthetic routes. Initially, commercially available fatty acid esters such as methyl palmitoleate (FA16:1), methyl oleate (FA18:1) and methyl eicosapentaenoate (FA20:5) were used as model compounds to study the reactions including a screening of different metathesis catalysts and to identify the products formed. Butenolysis (10-fold excess of 2-butene at -5 °C, 0.1 mol% HG2) of these model substrates yields short-chain unsaturated fatty acid methyl esters as well as short-chain mono- and dienes with conversions around 90% and high selectivities. Notably, FA20:5, a five-fold unsaturated fatty acid relatively abundant in the algae is successfully converted to four equivalents of 2,5-heptadiene. These olefinic products were further processed into value added linear mid-chain (di-)carboxylic acid esters via isomerizing alkoxy-carbonylation. Butenolysis and subsequent isomerizing alkoxycarbonylation was further performed on algae oil extracted from *Phaeodactylum tricornutum (vide supra)*. Hoveyda-Grubbs 2nd generation metathesis catalyst (HG2) appeared to be compatible with the multicomponent

mixture of numerous lipids and non-lipid compounds present in algae oil as no adverse effect on conversion and selectivity was observed. Thereby GC analysis revealed a virtually complete conversion of the unsaturated fatty acids (98% for the mono-unsaturated fatty acid and 100% for FA20:5). Subsequently, the reaction mixture of butenolysis of algae oil was subjected to the isomerizing alkoxycarbonylation without any intermediate purification step. After three days all butenolysis products were completely converted. Notably, the isomerizing alkoxycarbonylation catalyst was also compatible with remaining components present in algae oil such as polar diacylglycerides or chlorophylls and was not deactivated by residues of the quenched metathesis catalyst. For both reaction steps applying algae oil, high selectivities for the desired products were achieved.

All in all, this approach provides access to several carboxylic mono- and diacid esters of chain length ranging from C8 to C12 (such as azelaic acid ester, suberic acid ester and dodecanedioic acid ester), that are in demand but to which access is currently limited, fully based on algae oils as a renewable resource (**Figure 6-1**).

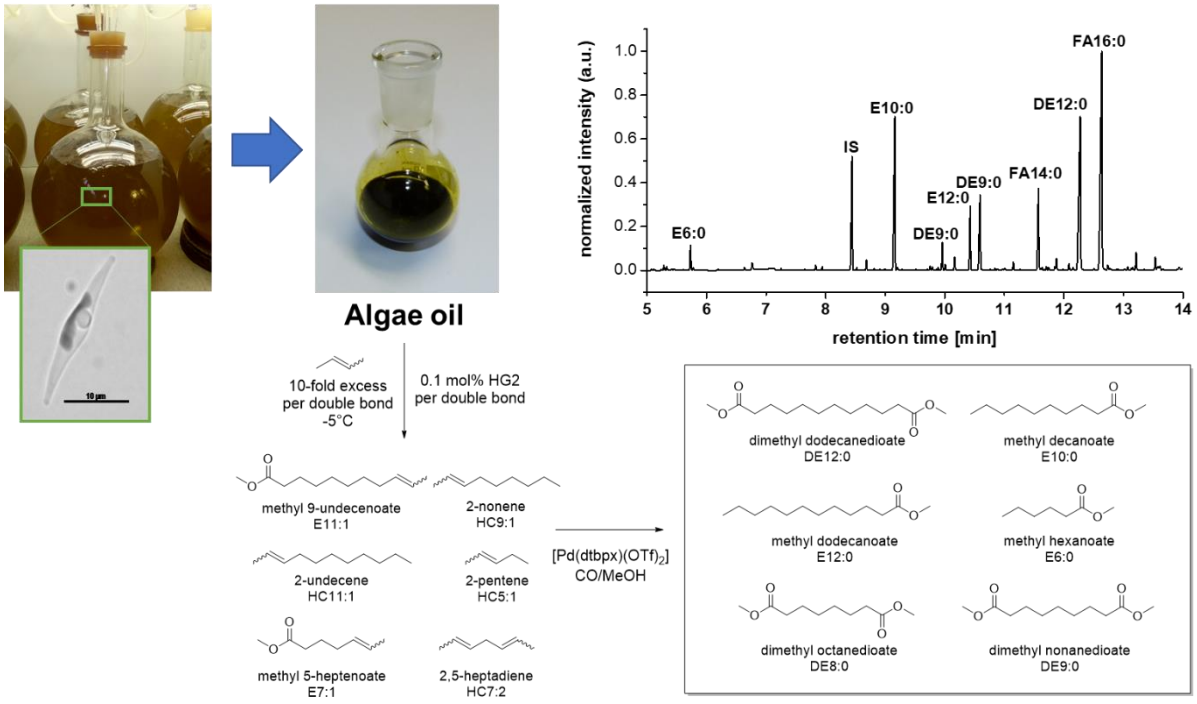

Figure 6-1: Reaction scheme and gas chromatogram of methoxycarbonylated butenolysis products of crude algae oil.

The resulting mono-carboxylic acid esters can serve as surfactants (E12:0 and E10:0) or food additives (E6:0), while dicarboxylic acid esters are useful as lubricants or monomers in polycondensation reactions. In particular the C9 diester azelaic acid ester (DE9:0) is of high interest, as current production requires the technically challenging and potentially hazardous ozonolysis. Moreover, the route presented in this thesis provides an additional approach to other

diesters (DE10:0 and DE12:0) and also a new, potentially interesting diester, DE8:0. All in all, the entire two-step catalytic route yields a number of desirable products by exploiting the entire fatty acid spectrum present in algae oil from *Phaeodactylum tricornutum*.

Besides butenolysis, also cross-metathesis with ethylene is a versatile tool to convert fatty acids into a wide spectrum of unsaturated compounds and gives access to products with terminal double bonds. In accordance to the experiments of single fatty acids as model substrates, the ethenolysis of crude algae oil, which was extracted via a modified Folch method from the diatom *Phaeodactylum tricornutum*, was performed using Hoveyda-Grubbs 1st generation catalyst (HG1) at 1.5 bar ethylene pressure and ambient temperature. Also, this metathesis catalyst was compatible with the multi-component mixture of the crude algae oil (**Figure 6-2**).

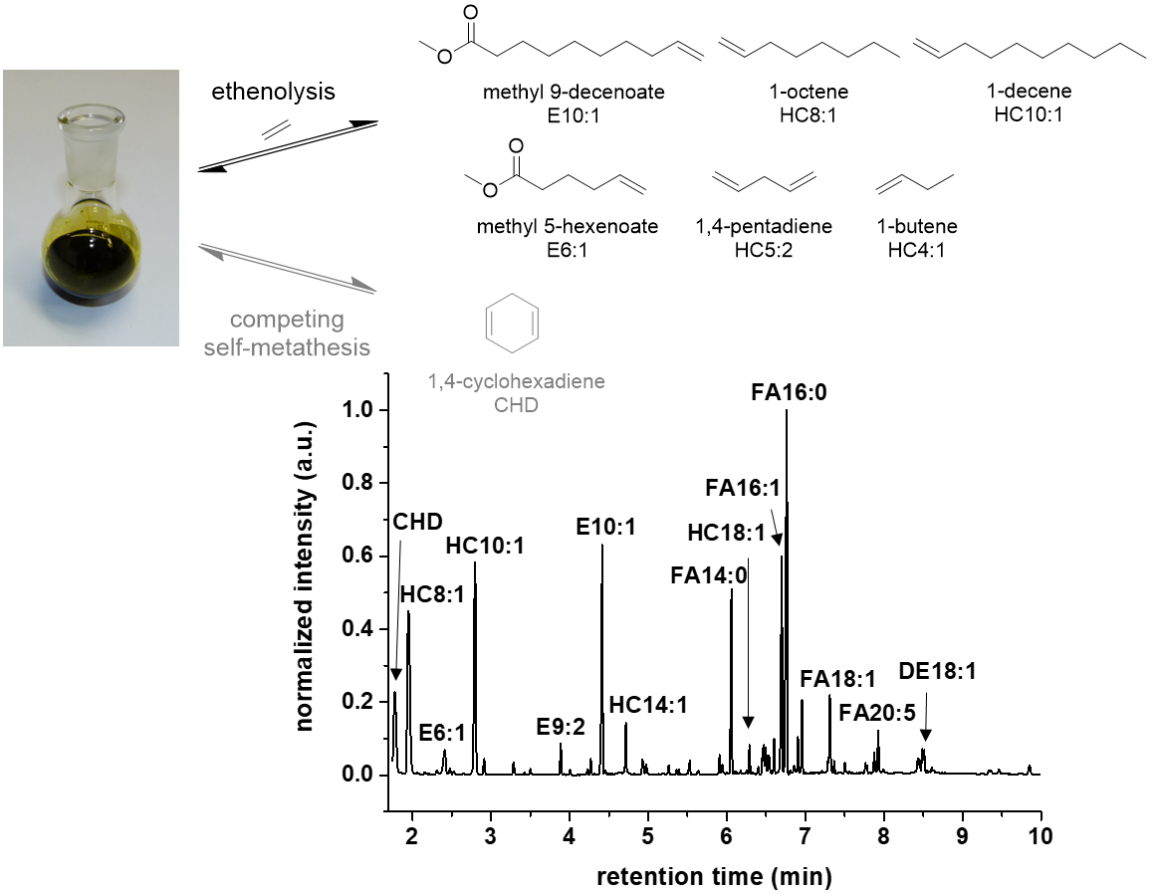

Figure 6-2: Gas chromatogram of the reaction mixture of ethenolysis of crude algae oil (after transesterification with methanol) with assignment of identified cross-metathesis products (1-octene (HC8:1), methyl 5-hexenoate (E6:1), 1-decene (HC10:1), methyl 5,8-nonadienoate (E9:2) and methyl 9-decenoate (E10:1)), self-metathesis products (1,4-cyclohexadiene (CHD), 7-tetradecene (HC14:1), 9-octadecene (HC18:1), dimethyl 9-octadecenedioate (DE18:1)), unconverted starting materials (FA16:1, FA18:1 and FA20:5) and saturated fatty acid methyl esters (FA14:0 and FA16:0).

For the mono-unsaturated fatty acids (FA16:1 and FA18:1) conversions of 67% and 69%, respectively, were observed, whereas the conversion of the five-fold unsaturated fatty acid

FA20:5 was even higher (89%). Via ethenolysis of the mono- unsaturated fatty acids mid-chain alkenes and unsaturated fatty acids with terminal double bonds can be produced in high selectivities.

Yet, in contrast to butenolysis, in the cross-metathesis with ethylene intramolecular self-metathesis of FA20:5 leading to 1,4-cyclohexadiene (CHD) was more pronounced. Besides the limited stability of the methylidene complex in contrast to its ethylidene analogue (formed in the butenolysis reaction), this higher selectivity for 1,4-cyclohexadiene can be mainly ascribed to a significant lower ethylene concentration. Due to limited variety of fatty acids occurring in nature, the product spectrum available via ethenolysis is restricted and predominantly products with even number of carbon atoms are formed. However, via the combination of isomerization and ethenolysis the product scope can be broadened.

This approach of isomerizing ethenolysis was demonstrated for methyl oleate using Ru alkylidenes as metathesis catalysts and [Pd(dtbpx)(OTf)$_2$] as isomerization catalyst. As this work focusses on the selective formation of products with chain length below 10 carbon atoms, which are otherwise only accessible by thermal cracking of the fatty acid chain, a first chain shortening ethenolysis step is followed by isomerizing ethenolysis (**Figure 6-3**).

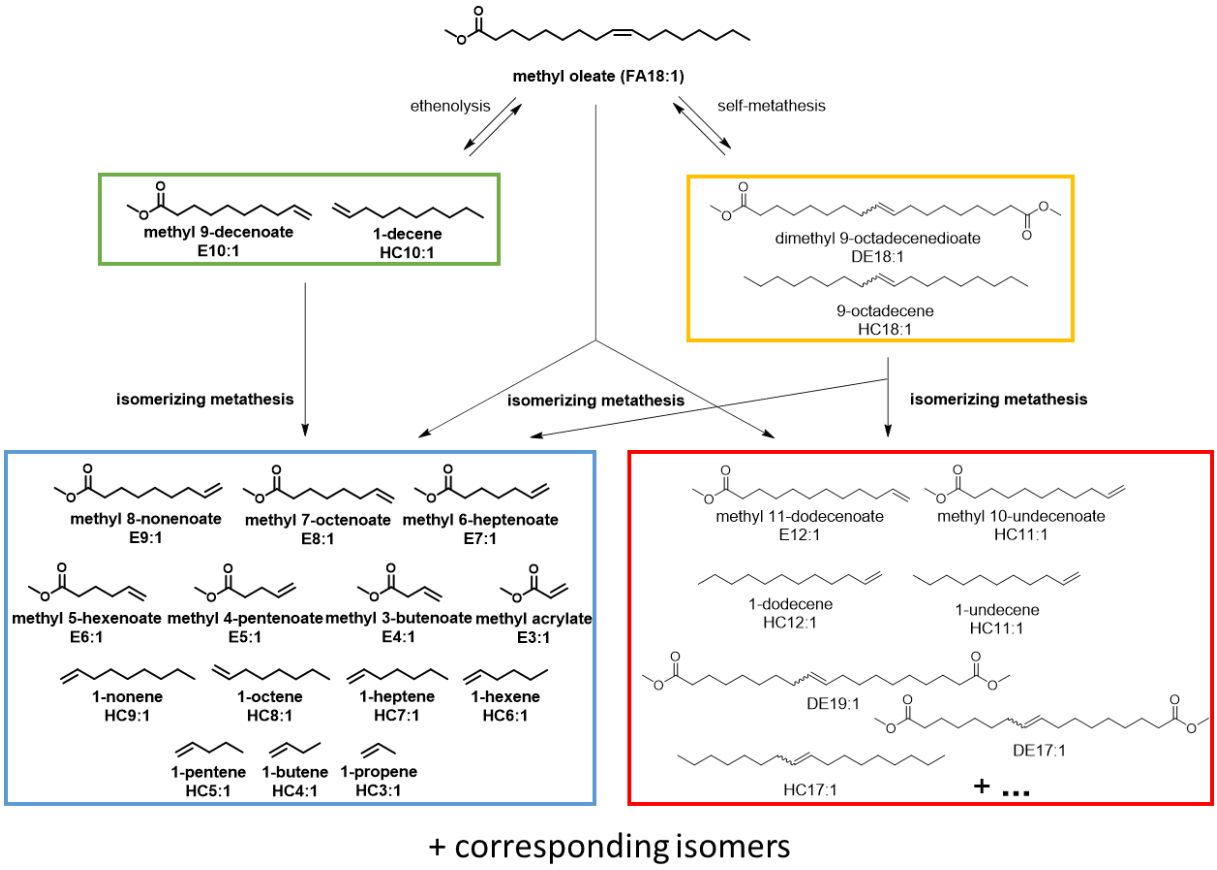

Figure 6-3: Possible products from isomerizing ethenolysis: primary ethenolysis products (green box), primary self-metathesis products (yellow box), products of isomerization and metathesis C<10 (blue box), products of isomerization and metathesis C>10 (red box).

This two-step approach (ethenolysis and subsequent isomerizing ethenolysis) was investigated with Hoveyda-Grubbs 1st and 2nd generation catalyst, however both catalysts were adversely affected by the applied [Pd(dtbpx)(OTf)$_2$]. In case of HG2 further addition of metathesis catalyst allowed the isomerized products for further conversion in metathesis leading to a broad spectrum of unsaturated products with different chain length. Experiments with HG1 could also be of interest.

So far, it was demonstrated that fatty acids from microalgae are attractive compounds for catalytic upgrading to chemicals, but their extraction often requires energy-intensive multi-step procedures and the use of various organic solvents. To relieve this bottleneck, a straightforward approach of combined extraction and catalytic functionalization via olefin cross-metathesis (ethenolysis and butenolysis) in supercritical CO_2 ($scCO_2$) was proposed. This is demonstrated for *Phaeodactylum tricornutum* microalgae biomass.

Initially, the $scCO_2$ extraction of lipids from *Phaeodactylum tricornutum* was investigated by varying pressure and temperature. In general, yields could be enhanced applying ultrasonic pre-treatment (**Figure 6-4**). Under optimum conditions (90 °C, 620 bar, $\rho(CO_2)$ = 0.90 g mL^{-1}) $scCO_2$ extracted the lipids selectively and quantitatively with a yield of 25 wt% from previously disrupted cells, while for organic solvent extraction a yield of 28 wt% was obtained. This difference can be explained by a higher selectivity for fatty acids in $scCO_2$ extraction compared to organic solvent extraction, which also extracts polar diacylglycerides and chlorophylls.

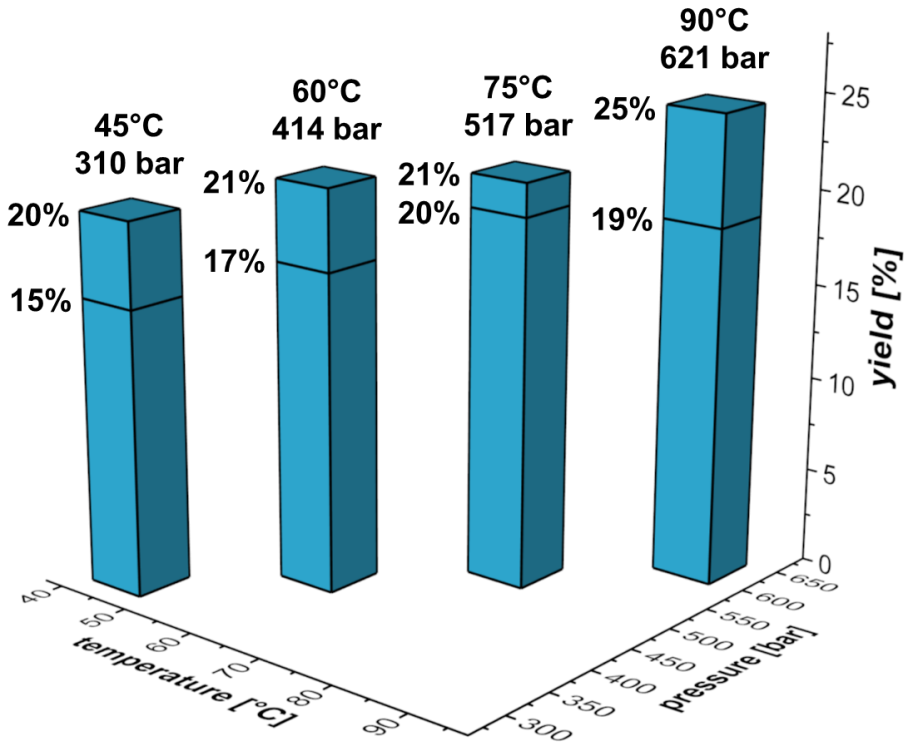

Figure 6-4: Yields of $scCO_2$ extraction at different pressures and temperatures at constant density (0.9 g mL^{-1}). Lower part of the bar: extraction of freeze-dried algae, lower and upper part of the bar: extraction of ultrasound pre-treated freeze-dried algae.

To study suitable reactions conditions for the combination of extraction and catalytic upgrading, methyl oleate was used as model compound and a mixture of fatty acid esters resembling the algae oil composition was investigated. At optimized conditions of 45 °C and a total pressure of 300 bar for the ethenolysis in $scCO_2$ Hoveyda-Grubbs 1st generation and for the butenolysis Hoveyda-Grubbs 2nd generation catalyst turned out to be suitable catalysts. The performance of each catalyst in $scCO_2$ is comparable to reactions in common organic solvents such as dichloromethane. Based on these results, ethenolysis and butenolysis, respectively, were also carried out with crude algae oil which was extracted separately with $scCO_2$ in advance. Thereby for both transformations high conversions and selectivities were achieved comparable to those obtained in the reactions in $scCO_2$ with the model substances or mixture thereof. Please note, that in this work catalyst loadings of typically 0.1 mol% were employed. This allows for conclusions on severe detrimental effects of algae oil components on catalyst stability or selectivity, even if commercial applications call for lower loadings.

Furthermore, in terms of reducing multiple reaction steps, avoiding solvent removal and integrating the extraction step into the valorization of the feedstock, a combined approach of extraction and cross-metathesis of microalgae in $scCO_2$ was demonstrated (**Figure 6-5**). By this one-pot approach, the mid-chain olefin and unsaturated ester products were directly accessible from the algal biomass. The product spectrum obtained compares to alkenolysis of individual

model compounds in $scCO_2$ as well as of separately $scCO_2$ extracted microalgae oil. In addition to its advantageous selectivity as a solvent, CO_2 is benign and easy to remove from the products. All in all, this combination of extraction and catalytic upgrading of lipids can help to overcome the bottleneck biomass extraction represents for its utilization as a feedstock, and of microalgae in particular.

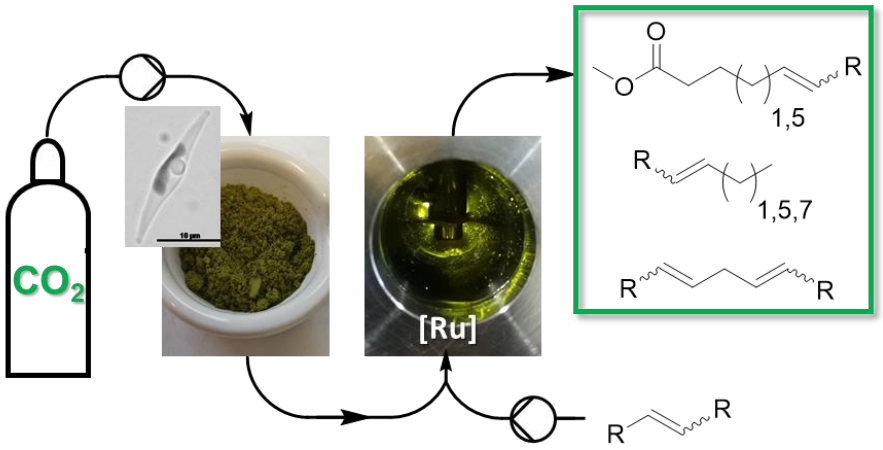

Figure 6-5: Schematic process design of extraction and catalytic valorization of microalgae in $scCO_2$ to mid-chain olefins and unsaturated esters.

7 Appendix

Table 7-1: 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*
Methyl nonanoate	E9:0	0.69	0.69
Methyl 5,8-decadienoate	E10:2	0.74*	0.69
2,5-Octadiene	HC8:2	0.66*	1,02*
2,5,8-Decatriene	HC10:3	0.83*	1,04*
Dimethyl octanedioate	DE8:0	0.52	0.43
Dimethyl nonanedioate	DE9:0	0.57	0.45
Dimethyl dodecanedioate	DE12:0	0.95	0.63
Methyl decanoate	E10:0	0.73	1.09
Methyl dodecanoate	E12:0	0.95	0.76
Heptanone		0.52	0.76
Methoxyester		0.55*	0.50*

*Derived from comparable compounds.

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