Cross-Metathesis of Microbial Oils for the Production of Advanced Biofuels and Chemicals

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ABSTRACT: A range of microbial oils were cross-metathesis-sized with ethene using Hoveyda–Grubbs second-generation catalyst. The products formed from the microbial oils were compared to alternative first- and second-generation oils. Upon separation, three separate fractions were produced: an alkene hydrocarbon fraction or aviation fuel fraction (AFF), a shorter-chain triglyceride fraction that upon transesterification was suitable as a road transport fuel (road transport fraction, RTF), and a volatile short-chain alkene fraction (gas phase fraction, GPF). The fuel fractions were purified through distillation and compared to the relevant fuel standards. Though there was variation for the RTF because of the presence of long-chain saturates, all the RTF produced fell within the ASTM standard for biodiesel. The AFF was found to be highly suitable for aviation, falling entirely within the DEF-STAN fuel standard. In addition, the AFF possessed an energy density higher than that of Jet A-1, whereas 1-decene was found to have an oxidative stability higher than that of jet fuel. Finally, the GPF was found to predominantly contain propene, butene, and pentadiene isomers, all of which have application in the polymer industry. With further development, this process could provide the basis for a microbial oil biorefinery for the production of sustainable biofuels and polymer precursors.

KEYWORDS: Metathesis, Aviation, Biofuel, Biorefinery, 1-Decene, Jet

INTRODUCTION

Currently, biodiesel is predominantly produced from terrestrial crop oils via transesterification. However, a number of technical issues associated with biodiesel limit the current blend level in diesel, being entirely unsuitable for aviation where the presence of FAME is capped at 5 ppm in the Jet A-1 standard. Biodiesel has a lower energy density because of the presence of oxygen, poor low-temperature properties because of the long carbon chain length, and poor oxidative stability because of its typical polyunsaturated components.

Furthermore, the vast majority of biodiesel is sourced from first-generation feedstocks, which compete with food production while only supplying a fraction of the current demand. As such, current research efforts have mainly focused on the development of alternative oil sources. These include second-generation sources such as waste cooking or tallow oils or so-called third-generation feedstocks such as microalgal and yeast oils. Microbial oils have the potential to produce significantly higher amounts of oil compared to their terrestrial counterparts. However, biodiesel produced from microalgal sources is extremely variable and can be highly polyunsaturated, which would not align with ASTM D6751 standards. Yeast oils tend to have a smaller number of fatty acids, similar to terrestrial oils, for example, Rhodoturula glutinis produces oil with properties similar to rapeseed or palm oil depending on the environmental conditions and as such would have similar performance issues that limit the applicability. A key sustainability goal is therefore to develop alternative chemical tranformations of oils that produce fuels with physical properties superior to biodiesel and that can be used in higher blend levels.

One alternative to transesterification is the olefin metathesis reaction, a key chemical transformation used in many oil-based industrial processes to produce polymers and high-value chemicals. The majority of metathesis research has focused on oil self-metathesis, whereby the double bonds of the
fatty acid chains exchange with each other to produce oligomers.17,18 Alternatively, oil cross-metathesis, whereby an external alkene source is used to cleave the double bonds present in triglycerides, can yield shorter-chain compound,19,20 potentially more suited as drop-in fuels.

Ruthenium alkylidene-based metathesis catalysts, such as Grubbs first-generation catalyst and subsequent generations, have the best functional group tolerance, and some do not require an inert atmosphere.21 The majority of metathesis research focuses on these species and related catalyst systems. High turnover numbers at low catalyst loadings have been reported by Patel et al. for the cross-metathesis of methyl oleate (as high as $4.7 \times 10^3$, with a substrate–catalyst ratio of 1:5 $\times 10^3$) with short internal alkenes such as 2-butene and 3-hexene.22 In the same study, the cross-metathesis of chemical-grade glyceryl trioleate as well as natural triglycerides with butene were attempted. It was found that the turnover for glycerol trioleate was lower than that reached for methyl oleate ($9.3 \times 10^3$), which decreased further upon using natural oils ($3.8 \times 10^4$ for sunflower oil, $3.7 \times 10^4$ for canola, $2.4 \times 10^4$ for soya, and $2.3 \times 10^4$ for linseed). Though no specific explanation was given for this decrease, it is likely due to the relative number of double bonds as well as the small amount of impurities found in natural oils.

However, the short-chain alkenes used in these reactions are derived from petrochemicals. Ideally, the external alkene source would be derived from renewable sources, such as ethene produced by dehydration of bioethanol.23 Cross-metathesis using ethene (ethenolysis) is far less favored because of the instability of the intermediate ruthenium methylidenes.24 This instability, for early iterations of Grubbs catalyst, leads to turnover numbers (TON) magnitudes lower than those reported for internal alkenes (0.6–7.8 $\times 10^3$)25 and high yields only achievable with higher catalyst loadings (3.4–4.9 mol%).26 Much work has been done in recent years to improve the conversion and TONs of carbene-based ruthenium catalysts for plant oil ethenolysis, such as altering the N-heterocyclic portion of the catalyst (reaches TONs > $2.0 \times 10^3$)19 and by producing phoban–indenylidene complexes (reported to reach TON = $7.2 \times 10^3$).26 Efficient ethenolysis, reaching conversions as high as 89%, has also been reported in room-temperature imidazolium-type ionic liquids, in an attempt to improve the recyclability of the homogeneous catalysts.27

Previously, research has been carried out on fatty acid alkyl ester (FAAE) model compounds (normally methyl oleate), allowing for easier analysis and preventing oligomer formation from oil self-metathesis. Because of the common distribution of the double bonds present in polyunsaturated fatty acid chains, metathesis of these oils leads to the formation of more complex products such as a range of volatile alkenes, such as 1-butene, 1,4-pentadiene, and 3-hexene.28–30 These components have the potential to be useful precursors for higher-value products or as co-monomers for polymer production.31–33

Although improvement and development of the ethenolysis catalysis system is important, determining the viability of the products achieved from such reactions for their applicability as fuels is vital. To date, only a handful of reports have considered metathesis as a potential process for the production of fuels,34–37 and no reports have investigated the suitability of microbial oils for cross-metathesis. In this study, the cross-metathesis of ethene and microbial oils was examined and compared with those of first- and second-generation oils. The fractions produced from the metathesis were then examined for their applicability as advanced biofuels and products.

**Experimental Methods**

**Materials.** All solvents were purchased from Fisher (U.K.), were reagent-quality, and were dried by passing through anhydrous alumina columns using an Innovative Technology Inc. PS-400-7 solvent purification system unless otherwise stated. Deuterated solvents ([CDCl$_3$, D$_2$O] were purchased from Fluorochem, U.K. Sulfuric acid, Hoveyda–Grubbs second-generation catalyst ([1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene] dichloro (phenylmethylene) (tri-cyclohexylphosphine) ruthenium, HGII), triethylamine, and tris( hydroxymethyl)phosphine were all obtained from Sigma-Aldrich, U.K., and used without further purification. Rapeseed oil, sunflower oil, and fresh coffee grounds were purchased from a local supermarket and used without further purification. Ethene (research-grade, >99.99% purity) was obtained from BOC Ltd., U.K.

**Methods. General Analysis.** Analysis of the oils and reaction mixtures was carried out by $^1$H NMR spectroscopy and GC-MS. $^1$H NMR spectroscopic measurements were carried out at 298 K using a Bruker AV300 spectrometer, operating at 300.13 MHz for $^1$H. Typically, samples were analyzed in CDCl$_3$, and spectra were referenced to the residual CHCl$_3$ peak from the solvent ($\delta$ 7.26 ppm). GC-MS analysis was carried out using the Agilent 7890B Gas Chromatograph equipped with a capillary column (30 m $\times$ 0.25 mm internal diameter) coated with DB-FFAP (nitrogenphosphoric acid modified polyethylene glycol), using stationary phase (0.35 μm film thickness) and a He mobile phase (flow rate = 1.2 mL min$^{-1}$), coupled with an Agilent 5977A inert MSD with Triple Axis Detector. Approximately 50 mg of each sample was dissolved in 10 mL of ethyl acetate or 1,4-dioxane, and 1 μL of each solution was loaded onto the column, preheated to 40 °C. This temperature was held for 1 min, and the column was then heated to 250 °C at a rate of 20 °C min$^{-1}$ and then held for 10 min.

**Microbial and Waste Coffee Oil Production.** The coffee oil was extracted and purified using our previously reported method.38 Oil purity was determined by $^1$H NMR spectroscopy and was found to be >99% triglyceride. Strains of Rhodotorula glutinis (NCYC 2439) and Rhodotorula minuta (NCYC 62) were purchased from the National Collection of Yeast Cultures (Norwich, U.K.) and cultured according to previously reported methods.39 Pseudochoristis ellipsospora was cultured in a 500 L raceway pond using minimal media over 20 days. Scenedesmus obliquus strain CCAP 276/7 was cultured using 1% (v/v) FEAF (nitrogenphosphoric acid modified polyethylene glycol), using stationary phase (0.35 μm film thickness) and a He mobile phase (flow rate = 1.2 mL min$^{-1}$), coupled with an Agilent 5977A inert MSD with Triple Axis Detector. Approximately 50 mg of each sample was dissolved in 10 mL of ethyl acetate or 1,4-dioxane, and 1 μL of each solution was loaded onto the column, preheated to 40 °C. This temperature was held for 1 min, and the column was then heated to 250 °C at a rate of 20 °C min$^{-1}$ and then held for 10 min.

**Oil/Ethene Cross-Metathesis.** For the metathesis reactions, a Fisher Porter pressure reaction vessel, with sample ports and attachments for connection to a Schlenk line (picture and schematic in the Supporting Information) was used. All reactions were carried out under inert conditions using standard Schlenk line techniques under a dry Ar atmosphere. In all reactions, the triglyceride source (1.0 mL, 0.91 g, 0.0010 mol) was added to the Fisher Porter bottle and dried and degassed under vacuum. Hoveyda–Grubbs second-generation catalyst (0.032 g, 5.1 $\times$ 10$^{-5}$ mol) was added to a Schlenk tube and dried under vacuum. Dry DCM (15 mL) was added to the Schlenk tube to solubilize the catalyst. The solution was immediately transferred to the Fisher Porter bottle and the reaction mixture heated (60 °C), pressurized with ethene (10 bar), and stirred (1200 rpm). After a reaction period of 1 h, the resulting mixtures were purified according to the literature method given by Maynard and Grubbs,40 whereby the reaction mixture was added to a solution of triis(hydroxymethyl)phosphine ([P(CH$_2$OH)$_3$]$_3$g 0.0010 mol) and triethylamine (Et$_3$N, 0.014 mL, 0.010 g, 1.0 $\times$ 10$^{-5}$ mol) in DCM (20 mL) and stirred for 10 min. Distilled water (30 mL) was then added and the biphasic solution vigorously stirred for >15 min before being allowed to separate. The aqueous layer was removed, and the organic layer was...
washed further with distilled water (2 × 30 mL) before removing the solvent in vacuo to isolate the products.

**Distillation of Aviation Fuel Fraction.** The metathesis product mixture was distilled using a Schlenk vacuum and liquid-N$_2$ trap. Under inert conditions, the metathesis product mixture was heated to 120 °C and subjected to reduced pressure for 1 h. After this time, the pressure in the vessel was allowed to equilibrate and the Schlenk line was allowed to reach ambient pressure before the liquid N$_2$ was removed from the trap and allowed to reach ambient temperature. The resulting condensate was transferred to a 100 mL round-bottomed flask with DCM washings, which was subsequently removed in vacuo at room temperature.

**Production of Road Transport Fraction.** The residue remaining after the short-chain alkenes had been removed was added to an excess of methanol (∼25 mL) and sulfuric acid (10 wt % in relation to the residue). The reaction mixture was then refluxed for 24 h. On completion of the reaction period, the mixture was allowed to cool to room temperature before being washed with distilled water (4 × 30 mL) to remove the methanol, glycerol, and acid catalyst. The glyceride—fatty acid methyl ester (FAME) yield was calculated using $^1$H NMR spectroscopy to ensure that >99% of the glyceride species had been converted.

**Fuel Properties.** Where suitable, the viscosity was measured using Canon-Fenske capillary kinematic viscometer, in accordance with ASTM D445 standard. Temperature modulation was achieved using a refrigeration/heating unit. Samples within the viscometer were allowed to rest at 40 or −20 °C as appropriate for a minimum of 5 min prior to viscosity measurement to allow temperature equilibration. For the oils and highly viscous products, a Bohlin C-VOR digital rheometer was used. The rheometer used was of the "cone and plate" variety, whereby the fluid is placed on a plate and a shallow cone (in this case, 1°) is lowered onto it. Subjecting the fluid to a specific shear stress, the dynamic viscosity was determined and converted to kinematic viscosity using the density.

Four points of the fuels were determined visually by cooling of approximately 0.5 mL samples in a digitally controlled low-temperature freezer set to specific temperatures. The samples were allowed to rest at each temperature for a minimum of 60 min in order to allow equilibration, after which the samples were checked in order to see if their pour point had been reached. Energy densities were measured using the IKA C1 static-jacket oxygen bomb calorimeter, in accordance with DIN 51900 and ISO 1928. Sample sizes of 0.3 g were used for each test and repeated three times.

### RESULTS AND DISCUSSION

A range of microbial oils from both heterotrophic and photoautotrophic organisms were screened for their viability

Table 1. Composition of Liquid Metathesis Products As Determined by GC-MS

|               | rapeseed metathesis products | sunflower metathesis products | coffee metathesis products | P. ellipsoidea metathesis products | S. obliquus metathesis Products | R. glutinis metathesis products | R. minuta metathesis products | M. pulcherrima metathesis products |
|---------------|-----------------------------|-------------------------------|---------------------------|------------------------------------|---------------------------------|--------------------------------|--------------------------------|----------------------------------|
| FAME (%)      | C8:1                        | 0.0                           | 0.0                       | 0.0                                | 2.3                             | 1.0                            | 0.0                            | 0.0                              |
|               | C9:1                        | 1.0                           | 0.8                       | 0.0                                | 0.3                             | 2.1                            | 0.3                            | 0.7                              |
|               | C10:1                       | 54.4                          | 56.3                      | 12.5                               | 55.8                            | 25.0                           | 42.3                           | 21.0                             |
|               | C11:1                       | 1.3                           | 1.1                       | 0.0                                | 0.3                             | 0.0                            | 0.5                            | 0.0                              |
|               | C12:1                       | 3.6                           | 0.9                       | 4.3                                | 2.5                             | 3.2                            | 0.3                            | 0.0                              |
|               | C14:0                       | 0.0                           | 0.6                       | 0.0                                | 1.9                             | 3.6                            | 1.2                            | 2.1                              |
|               | C15:1                       | 1.9                           | 4.3                       | 1.5                                | 0.0                             | 2.8                            | 1.9                            | 1.8                              |
|               | C16:0                       | 7.5                           | 8.8                       | 51.0                               | 0.0                             | 36.7                           | 24.4                           | 21.2                             |
|               | C16:1                       | 2.3                           | 1.4                       | 6.2                                | 1.6                             | 2.4                            | 0.2                            | 0.0                              |
|               | C17:0                       | 0.0                           | 0.0                       | 0.0                                | 0.0                             | 0.0                            | 0.4                            | 0.0                              |
|               | C17:1                       | 0.0                           | 0.0                       | 0.0                                | 0.0                             | 0.0                            | 0.0                            | 0.0                              |
|               | C18:0                       | 2.4                           | 5.0                       | 10.4                               | 12.6                            | 3.7                            | 6.0                            | 5.2                              |
|               | C18:1                       | 6.0                           | 3.5                       | 0.7                                | 0.5                             | 4.4                            | 4.6                            | 8.3                              |
|               | C18:2                       | 0.0                           | 1.0                       | 0.2                                | 0.0                             | 0.0                            | 0.7                            | 0.0                              |
|               | C20:0                       | 0.0                           | 0.4                       | 3.4                                | 1.0                             | 1.0                            | 0.4                            | 0.0                              |
|               | C20:1                       | 0.0                           | 0.0                       | 0.0                                | 0.0                             | 0.0                            | 0.0                            | 0.0                              |
|               | C22:0                       | 0.0                           | 0.9                       | 0.7                                | 0.0                             | 1.5                            | 0.3                            | 0.0                              |
| total (%)     | 80.4                        | 85.1                         | 90.9                      | 78.7                               | 87.4                            | 83.4                           | 60.2                           | 75.3                             |
| olefin (%)    | C10:1                       | 4.7                           | 4.0                       | 3.9                                | 0.7                             | 0.0                            | 3.9                            | 1.8                              |
|               | C11:1                       | 0.5                           | 0.2                       | 0.0                                | 0.0                             | 0.0                            | 0.0                            | 0.0                              |
|               | C12:1                       | 0.8                           | 1.3                       | 0.2                                | 0.7                             | 0.7                            | 0.2                            | 0.0                              |
|               | C13:1                       | 0.0                           | 0.0                       | 0.0                                | 0.0                             | 0.0                            | 0.0                            | 0.0                              |
|               | C14:1                       | 0.0                           | 0.0                       | 0.0                                | 0.0                             | 0.0                            | 0.0                            | 0.0                              |
|               | C15:1                       | 2.3                           | 2.3                       | 0.3                                | 0.8                             | 0.0                            | 1.0                            | 1.5                              |
|               | C16:1                       | 0.0                           | 0.2                       | 0.0                                | 0.0                             | 0.0                            | 0.0                            | 0.0                              |
|               | C17:1                       | 0.0                           | 0.0                       | 0.0                                | 0.0                             | 0.0                            | 0.0                            | 0.0                              |
|               | C18:1                       | 4.8                           | 1.5                       | 0.0                                | 2.8                             | 1.1                            | 1.1                            | 2.0                              |
| total (%)     | 13.1                        | 9.5                           | 4.4                       | 5.1                                | 1.7                            | 6.2                            | 5.2                            | 0.6                              |
| C18:1 FADME (%) | 4.3                          | 4.4                           | 1.1                       | 5.7                                | 0.0                            | 2.7                            | 4.2                            | 2.7                              |
| other (%)     | 0.7                          | 1.0                           | 3.5                       | 10.5                               | 10.9                           | 7.7                            | 30.3                           | 20.6                             |

$^a$Assigned “CX:Y”; X denotes carbon number and Y denotes number of double bonds. Different molecular species that fall within the same label according to these rules (i.e., 1-decene and 2-decene) are grouped together. FADME = fatty acid dimethyl ester.
as feedstocks in the metathesis ethenolysis reaction. The microbial oils investigated were compared to two first- or second-generation biodiesel feedstocks: rapeseed oil (high in monounsaturates) and sunflower oil (high in polyunsaturates). Triglyceride oil extracted from waste coffee grounds was also screened because of its potential as a suitable second-generation resource. The microbial oils selected were from two species of microalgae: *Pseudothecostis ellipsoidea*, an acidophilic oil producer, and *Scedesmus obliquus*, currently used in wastewater treatment and previously reported as having potential for oil production. These were compared to oils from *Rhodotorula glutinis* and *Rhodotorula minuta*, both oleaginous yeasts identified as having potential for biodiesel production, and *Metschnikowia pulcherrima*, an oleaginous yeast that can be cultivated under nonsterile conditions on a range of waste resources. The fatty acid profile of the oils used is given in the Supporting Information.

Although the glycerides are the predominant component of oils, a range of other biological compounds are present in the oils screened. H NMR was used to assess the purity of the glyceride oils investigated (see Supporting Information). Unsurprisingly, the edible oils, rapeseed and sunflower, almost exclusively contain triglycerides. Coffee oil and both *Rhodotorula* species are similarly pure. In the spectra of *P. ellipsoidea*, *S. obliquus*, and *M. pulcherrima*, however, there are a number of peaks not assignable to triglycerides. This is likely due to other biological oil materials such as sterols, cell residue, and phospholipids. These compounds could potentially affect the catalyst activity, the downstream processing, and the fuel properties of the resulting fuels.

**Metathesis Conversions and Products.** The oils were used as a substrate for cross-metathesis with ethene (10 bar), using Hoveyda–Grubbs second-generation catalyst (HGII), for 1 h at 60 °C. To assess products formed during the metathesis reaction, the samples were analyzed by GC-MS (Table 1). The reaction for those oils possessing a large proportion of unsaturates yielded a majority of monounsaturated C₁₀ esters, accounting for over half of the esters present in the rapeseed, sunflower, and *P. ellipsoidea* sample composition. This is expected because most unsaturates, though they may possess more than one double bond, have a double bond in the C₉ position and are saturated up to the ester moiety. However, although the vast majority of these C₁₀ monounsaturated esters were terminal alkenes (>90%), a number of alternative peaks associated with this species were also identified. Because of the lack of stereochemistry in terminal alkenes, it can be inferred that isomerization is taking place, moving the double bond up the carbon chain. This is typical of ruthenium catalysts in their hydride form, a likely decomposition product of HGII.

Isomerization in metathesis has been widely reported as a side-reaction in ruthenium–carbene-catalyzed metathesis as well as being the strongly preferred or even exclusively observed pathway because of the in situ formation of ruthenium hydride species. The isomerization associated with fatty acid ethenolysis using ruthenium–carbene catalysts produces unwanted internal alkenes that are difficult to separate via standard purification techniques and is the main limitation of its industrial application where terminal compounds specifically are needed. Although some methods have been developed to inhibit this isomerization, there may be little need to inhibit the isomerization in this case. The presence of internal alkenes should have a minimal effect on the general fuel properties of the molecules, and the stability of the fuel could be increased because terminal alkenes are generally more reactive as a result of their inherent lack of steric hindrance. This isomerization is potentially the reason for the presence of fatty acids and alkenes that are otherwise unexpected, such as C₉ and C₁₁ fatty acid alkenes and C₁₁ and C₁₂ alkenes.

In all the reaction mixtures, there were small amounts of unreacted monounsaturated fatty acids, which was expected because of the equilibrium associated with Ru-based metathesis catalysts at lower pressures. However, the polyunsaturates were significantly decreased because of the probability of each double bond being consumed, along with the potential production of 1,4-pentadiene, which would partition into the gas phase, thus significantly reducing the rate at which it would react. The lack of polyunsaturates would also increase the oxidative stability of any fuel produced by this method. Because of their lack of double bonds, saturated fatty acid chains were also present in the final reaction mixtures. Interestingly, a dimethyl ester (octadec-9-enedioic acid methyl ester) was observed in up to 5.7% of the final composition. This is most likely produced in one of three ways: between two C₁₈ fatty acid chains with a double bond in the C₉ position, on either the same or different triglyceride species, as the product of two terminally unsaturated C₁₀ fatty acids (produced from the reaction of the triglyceride with ethene) reacting with each other, or between one terminally unsaturated C₁₀ fatty acid and one C₁₈ fatty acid chain with a double bond in the C₉ position.

Five olefins were detected by GC-MS in the product mixtures analyzed, with 1-decene being the most prevalent. The light olefin fraction was separated through distillation under vacuum. It should be noted, however, that the metathesis of *S. obliquus* did not yield any 1-decene, and only dodecane is present. This is likely to be 6-dodecene, produced from the metathesis of two ω-6 fatty acids. Therefore, because of the lack of 1-decene in both *S. obliquus* and *M. pulcherrima*, presumably the cross-metathesis reaction between their triglycerides and ethene is not favored. Other olefins present in all but *S. obliquus* and *M. pulcherrima* are pentadecene (the likely cross-metathesis product between ω-6 and ω-9 fatty acids) and octadecene (the likely cross-metathesis product between two ω-9 fatty acids).

Because of the lack of short-chain alkenes present in the metathesis products, *S. obliquus* and *M. pulcherrima* oils were not suitable as metathesis fuel feedstocks and were not examined further. Because of the higher yields, *R. glutinis* was selected over *R. minuta* as the most suitable oleaginous yeast for further study. The metathesis product mixtures were then separated into three fractions. The gas fraction was collected and the composition analyzed by GC-MS. The lower boiling alkenes, potentially suitable as a jet fuel replacement, were collected through distillation (aviation fuel fraction, AFF), leaving behind the metathesis residue. After fuel analysis, this residue was transesterified to produce a mixture of FAME and higher-boiling-point alkenes (road transport fraction, RTF). The compositions and fuel properties of each fraction were analyzed (Figure 1).

**Road Transport Fraction.** The composition of the RTF is given in Table 2. No oxidation products were formed during the distillation of the C₁₂ and shorter olefins, demonstrating the stability of the mixtures. All the RTFs formed were a mixture of short-chain monounsaturated esters, saturated esters, and longer chain olefins.
Fuel Properties. All the oil feedstocks tested had a viscosity of above 20 mm² s⁻¹ at 40 °C (Figure 2); this is in general agreement with reported values. Sunflower oil exhibited the lowest viscosity (21.9 mm² s⁻¹), presumably because of its high amount of polyunsaturates. Correspondingly, the viscosity of the oleic acid rich rapeseed and R. glutinis oils were slightly higher at 24.2 and 25.4 mm² s⁻¹, respectively. The highly saturated coffee oil had a higher viscosity than the yeast oils.

Table 2. Composition of the Road Transport Fraction (RTF) Formed via the Transesterification of the Residue Left after Removal of the Aviation Fuel Fraction As Analyzed by GC-MS

| FAME (%)   | rapeseed RTF | sunflower RTF | coffee RTF | P. ellipoides RTF | R. glutinis RTF |
|------------|--------------|---------------|------------|-------------------|-----------------|
| C8:1       | 0.2          | 0.0           | 0.0        | 2.5               | 0.0             |
| C9:1       | 2.3          | 0.7           | 0.3        | 0.0               | 0.7             |
| C10:1      | 51.4         | 60.7          | 35.6       | 39.2              | 40.8            |
| C11:1      | 2.4          | 0.9           | 0.0        | 0.0               | 1.0             |
| C12:1      | 4.1          | 0.8           | 0.3        | 2.0               | 0.3             |
| C14:0      | 0.0          | 0.0           | 0.0        | 0.0               | 0.5             |
| C16:0      | 7.1          | 9.8           | 45.1       | 21.8              | 26.6            |
| C16:1      | 0.8          | 0.3           | 0.6        | 1.1               | 0.4             |
| C17:0      | 0.0          | 0.0           | 0.0        | 0.0               | 0.4             |
| C17:1      | 1.2          | 0.1           | 0.0        | 0.0               | 0.0             |
| C18:0      | 2.4          | 6.0           | 9.9        | 9.2               | 6.7             |
| C18:1      | 7.7          | 4.3           | 0.9        | 7.2               | 5.2             |
| C20:0      | 0.8          | 0.4           | 3.0        | 0.6               | 0.4             |
| C20:1      | 0.5          | 0.2           | 0.0        | 0.0               | 0.1             |
| total      | 80.9         | 84.2          | 95.8       | 83.8              | 83.2            |

| olefin (%) | rapeseed RTF | sunflower RTF | coffee RTF | P. ellipoides RTF | R. glutinis RTF |
|------------|--------------|---------------|------------|-------------------|-----------------|
| C14:1      | 0.1          | 0.0           | 0.0        | 0.0               | 0.0             |
| C15:1      | 1.3          | 0.3           | 0.0        | 0.0               | 1.1             |
| C16:1      | 0.4          | 0.0           | 0.0        | 0.0               | 0.0             |
| C17:1      | 0.4          | 0.0           | 0.0        | 0.0               | 0.1             |
| C18:1      | 2.7          | 0.4           | 0.0        | 2.3               | 0.3             |
| total      | 4.9          | 0.7           | 0.0        | 2.3               | 1.5             |

| C18:1 FADME (%) | rapeseed RTF | sunflower RTF | coffee RTF | P. ellipoides RTF | R. glutinis RTF |
|-----------------|--------------|---------------|------------|-------------------|-----------------|
| 6.4             | 4.5          | 1.5           | 5.2        | 5.2               |

| other (%) | rapeseed RTF | sunflower RTF | coffee RTF | P. ellipoides RTF | R. glutinis RTF |
|-----------|--------------|---------------|------------|-------------------|-----------------|
| 7.8       | 10.6         | 3.6           | 8.7        | 10.4              |

“Assigned “CX:Y”; X denotes carbon number and Y denotes number of double bonds. Different molecular species that fall within the same label according to these rules (i.e., 1-decene and 2-decene) are grouped together. FADME = fatty acid dimethyl ester.
(31.1 mm² s⁻¹), though the viscosity of *P. ellipsoidea* oil was significantly higher at 109 mm² s⁻¹, presumably because of impurities present in the oil that possess strong intermolecular bonding. On metathesis of all the oils examined and after the short-chain alkenes were removed through distillation, the viscosity of the mixtures was remarkably similar to that of the parent oil. Presumably, though shorter chain FAMEs are present in the residue mixture, the distillation of the short-chain alkenes increases the proportion of saturates in the residue, leading to increased viscosities. This demonstrates that creating short-chain triglycerides is not enough to reduce the viscosity of the oil to required levels, and the additional transesterification step is indeed necessary to produce a suitable RTF.

On transesterification of the metathesis residue, the viscosity of the mixtures is substantially reduced, although *R. glutinis* RTF was the only sample that fell within the European standards for biodiesel (Figure 3a). Rapeseed and sunflower RTF fell below the minimum, and coffee and *P. ellipsoidea* RTF fell above the maximum. All samples analyzed fell within the ASTM biodiesel standard (range 1.9–6.0 mm² s⁻¹). All of the oils possessed similar energy densities on the range of 37.7–40.1 MJ kg⁻¹ (Figure 3b). Coffee, *R. glutinis* oil, and *P. ellipsoidea* oils had slightly lower energy densities (37.7 and 38.2 MJ kg⁻¹, respectively) than rapeseed and sunflower oil (39.7 and 40.1 MJ kg⁻¹, respectively), likely because of the slightly higher amount of oxygenated impurities. The metathesis residues were all lower in energy density than their parent oil because of the removal of short-chain hydrocarbons, thus decreasing the C/O and H/C ratios of the overall mixture. Distilling the lower-boiling hydrocarbons decreased C/O and H/C ratios and thus decreased energy density was observed for all metathesis residue samples. Transesterification of the metathesis residue to the RTF introduces three methyl groups (i.e., 9 C–H bonds) per triglyceride rather than a glyceryl moiety (i.e., 5 C–H bonds), which increases the energy density slightly. Interestingly, the RTF for *P. ellipsoidea* exhibits a higher energy density than its original oil, likely due to the amount of oxygenated impurities present in the oil that were lost in the extensive processing and workup. The energy densities for all RTF fractions were between 33.8 and 36.8 MJ kg⁻¹, which is lower than those of diesel and biodiesel at approximately 45 and 40 MJ kg⁻¹, respectively.

The pour point is an important fuel metric because the fuel must flow, down to the lowest operational temperature. The pour points of the oils, metathesis residue, and RTF were therefore assessed (Figure 3c). For the oils, a wide range of pour points was observed, from as low as −8 to −7 °C for rapeseed oil and to as high as 8–10 °C for *P. ellipsoidea* oil, likely due to the difference in saturation and impurities. The pour point of the metathesis residues were higher than the original parent oils, presumably because of the increased proportion of saturates. Interestingly, the metathesis residue for *P. ellipsoidea* gelled at room temperature. Transesterifying the residue led to the lowering of the RTF pour point to the same range or lower than that of the original oil in all cases. Rapeseed RTF had the lowest pour point range, between −10 and −8 °C, with coffee and *P. ellipsoidea* the highest at 4–6.5 °C. All the pour points for the RTF were either the same or only a slight improvement on the parent oil. As with the viscosity, the presence of short-chain esters is balanced by the increased level of saturates in the biodiesel; therefore, production of an RTF with improved low-temperature properties would require a feedstock low in saturates.

**Aviation Fuel Fraction.** The AFF was separated by distillation from the metathesis products under vacuum at 60 °C. The composition of the AFF was analyzed by GC-MS (Figure 4). The major product from the reaction was 1-decene, which increased to 58.1% of the distillate for co-distillation with Jet A-1 (20% AFF, 80% Jet A-1). These blends were designated RMD-20 (rapeseed oil metathesis distillate...
blended 20 vol % with jet-fuel), SMD-20 (sunflower oil metathesis distillate blended 20 vol % with Jet A-1), CMD-20 (coffee oil metathesis distillate blended 20 vol % with Jet A-1), RgMD-20 (R. glutinis oil metathesis distillate blended 20 vol % with Jet A-1), and PMD-20 (P. ellipsoidea oil metathesis distillate blended 20 vol % with Jet A-1). As a comparison, a representative model mixture (AFF model) of the distillates was made from blending the individual olefins (purchased commercially), using an approximate average of the AFFs produced (i.e., 80 vol % decene, 5 vol % undecene, and 15 vol % dodecene).

At 40 °C, the blending of AFF did not significantly alter the viscosity of the Jet A-1 (Figure 5). The viscosity of Jet A-1 used in this study was 1.17 mm² s⁻¹ at 40 °C, whereas the blends with 20 vol % distillate ranged from 1.12 mm² s⁻¹ (rapeseed) to 1.29 mm² s⁻¹ (coffee). The increased viscosity of the CMD-20 is presumably due to the higher proportion of dodecene. According to the Jet A-1 fuel standards, the fuel is required to have a viscosity of 8 mm² s⁻¹ or below at −20 °C. At this temperature, all samples tested were found to be significantly below this maximum. The blends with 20 vol % distillate ranged from 4.00 to 4.18 mm² s⁻¹, significantly lower than Jet A-1’s value of 4.68 mm² s⁻¹. This is unsurprising because 1-decene and the AFF model mixture have a considerably lower viscosity of 2.52 and 2.41 mm² s⁻¹, respectively. These lower viscosities are most likely due to the lower proportion of aromatics and long chain alkanes.

The Jet A-1 standard sets a minimum freezing point of −47 °C. The freezing point of the Jet A-1 used in this study was found to be −51 °C. The metathesis distillates were still liquid at this temperature whereas the blended distillates were all found to flow up to −51 °C (Figure 6a). Similarly the flash points of the Jet A-1 and all the 20% Jet A-1 blends were found to be 41 °C (Figure 6b), despite the flash points of 1-decene, 1-undecene, and 1-dodecene being substantially higher. The energy densities for the metathesis distillate mixtures, along with jet A-1, 1-decene, and the AFF model were also measured (Figure 6c). The AFF model and 1-decene exhibited the highest energy density at 47.0 and 46.9 MJ kg⁻¹, respectively, significantly higher than that of Jet A-1 (44.7 MJ kg⁻¹). This is presumably due to the aromatic proportion present in Jet A-1, significantly reducing the H/C ratio of the fuel. The energy densities for the distillate mixtures were lower than those of the AFF model and 1-decene and higher than that of Jet A-1, with a range between 45.2 and 46.0 MJ kg⁻¹. The values for all fuels were above the minimum set by ASTM and DEF STAN standards (42.8 MJ kg⁻¹).

The AFF produced contains a significant portion of terminal alkenes and a small portion of internal alkenes because of the isomerization activity of the deactivated metathesis catalyst. Though this is not seemingly an issue in terms of the fuel properties, the catalyst is a significant portion of the cost of fuel production, so its replacement/rejuvenation will add cost to the process. Ideally, the catalyst would be robust and selective for terminal alkene production. A high proportion of terminal bonds, however, may reduce the stability of the fuel because of their higher reactivity compared with internal double bonds. To assess the stability of a fuel with a high portion of terminal double bonds, technical-grade 1-decene was subjected to Rancimat conditions (110 °C, airflow = 10 L h⁻¹), and samples were taken regularly between 1 and 72 h and analyzed by ¹H NMR spectroscopy (Supporting Information). For the first 24 h of the experiment, little change was observed. Only after 48 h does the fuel begin to degrade. The peaks associated with the terminal double bond (δ 4.9 and 5.8 ppm) are noticeably decreased, and there is a range of peaks from 2.0–4.5 ppm that are indicative of a range of oxygenated species including alcohols and carbonyl species. At 72 h, the double bonds were almost completely consumed: from 2.0–4.5 ppm there are wide, overlapping peaks that suggest a range of oxygenated species had been formed. This demonstrates that 1-decene is stable at Rancimat conditions for at least 24 h, significantly
Oil metathesis distillate blended 20 vol % with Jet A-1; RgMD-20, P. ellipsoidea oil metathesis distillate blended 20 vol % with Jet A-1; PMD-MS (Figure 7). 1-Butene (from the cross-metathesis of ethene pressure was released through a gas trap and analyzed via GC-metathesized, and once the reaction time was complete, the produced in the gas phase fraction (GPF), the oils were changes after less than 1 h under the same conditions.

All experiments were repeated three times, with average and standard deviation represented by the error bars. RMD-20, rapeseed oil metathesis distillate blended 20 vol % with jet-fuel; SMD-20, sunflower oil metathesis distillate blended 20 vol % with Jet A-1; CMD-20, coffee oil metathesis distillate blended 20 vol % with Jet A-1; RgMD-20, R. glutinis oil metathesis distillate blended 20 vol % with Jet A-1; PMD-20, P. ellipsoidea oil metathesis distillate blended 20 vol % with Jet A-1.

Figure 6. (a) Freezing point of Jet A-1, 1-decene, 1-undecene, 1-dodecene, AFF model, and all metathesis distillate blends, showing the maximum allowed value outlined in ASTM and DEF STAN standards (horizontal dashed line). (b) Flash points of Jet A-1, 1-decene, 1-undecene, 1-dodecene, AFF model, and all metathesis distillate blends, showing the minimum outlined in ASTM and DEF STAN standards (horizontal dashed line). (c) Energy densities of Jet A-1, 1-decene, AFF model, and all metathesis distillate blends, showing the minimum outlined in ASTM and DEF STAN standards (horizontal dashed line). All experiments were repeated three times, with average and standard deviation represented by the error bars. RMD-20, rapeseed oil metathesis distillate blended 20 vol % with jet-fuel; SMD-20, sunflower oil metathesis distillate blended 20 vol % with Jet A-1; CMD-20, coffee oil metathesis distillate blended 20 vol % with Jet A-1; RgMD-20, R. glutinis oil metathesis distillate blended 20 vol % with Jet A-1; PMD-20, P. ellipsoidea oil metathesis distillate blended 20 vol % with Jet A-1.

Oils from first-generation (rapeseed and sunflower), second-generation (waste coffee oil), and third-generation (microalgae and yeast) feedstocks were tested for their suitability as feedstock for the production of fuels and chemicals using olefin metathesis. The oils screened contained a wide range of fatty acids, differing largely in saturation. Oil from rapeseed, sunflower, P. ellipsoidea, and R. glutinis all achieved terminal-bond selectivity similar to that of the model glyceride components (35–40%), though M. pulcherima and S. obliquus performed worse presumably because of their higher saturation or impurities in the oil deactivating the catalyst.

The metathesis products of rapeseed, sunflower, coffee, P. ellipsoidea, and R. glutinis separated into the olefin aviation fuel fraction, AFF, and metathesis residue, which was then transesterified to give the road transport fraction, RTF. On metathesis of the oil and removal of the hydrocarbons produced, the energy density of the mixture was reduced compared to the parent oils. Transesterification this residue to produce the RTF increased the energy density slightly, ranging between 33.8 and 36.8 MJ kg⁻¹. Although this is still lower than conventional biodiesel, the improved stability and good low-temperature performance could potentially increase the viability. The pour points of the RTF produced were comparable to those of conventional biodiesel, where the kinematic viscosity was found to be within the limits set in the ASTM biodiesel standards. The AFF produced was a mixture of C₁⁰−C₁₂ alkenes, which proved to be highly promising fuels for the aviation sector. Despite the isomerization and presence of C₁₂ alkenes, the viscosity of the AFF–Jet-A1 blends and AFF model fell well within the maximum value for Jet-A1 and had a higher energy density than Jet A-1. Finally, the volatile gas fraction contained functional hydrocarbons that could have potential application as polymer precursors.

The metathesis of biologically sourced oils with ethene has excellent potential to produce multiple fuels from one oil feedstock while also producing valuable hydrocarbon side products for the polymer industry. With further development, the metathesis reaction could form the basis of a sustainable oil biorefinery.
Figure 7. Proportions of the short-chain alkenes found in the gas fractions of the metathesis reactions as analyzed by GC-MS and compared to known standards.

ASSOCIATED CONTENT

Supporting Information

Rig design, lipid profile of the original oils, 1H NMR spectra demonstrating the purity of the oils, and the GC-MS chromatographs of the GPF. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.b900228. The data generated over the course of this study can be freely accessed at http://dx.doi.org/10.15125/BATH-00083.

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Notes

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