

1 Decolorization Improves the Fuel Properties of 2 Algal Biodiesel from *Isochrysis* sp.

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1 ABSTRACT. Results from the comprehensive fuel testing according to American Society for
2 Testing and Materials International (ASTM) standards of an alkenone-free and decolorized
3 biodiesel produced from the industrially grown marine microalgae *Isochrysis* sp. are presented.
4 Fatty acid methyl ester (FAME) profiles of the non-decolorized and subsequently decolorized
5 biodiesel fuels were nearly identical, yet the fuel properties were remarkably different.
6 Significant positive impacts on the cetane number, kinematic viscosity, and lubricity were
7 observed, indicating a potential deleterious effect of pigments like chlorophylls and pheophytins
8 on these fuel properties. The decolorization process using montmorillonite K10 gave on average
9 90% mass recovery, and allowed for an otherwise unobtainable cloud point determination.
10 Oxidative stability of the decolorized *Isochrysis* biodiesel remained well below the minimum
11 prescribed in biodiesel standards due to elevated content of highly polyunsaturated fatty acids,
12 however other values were in the range of those prescribed in the ASTM standards. Overall,
13 decolorization improved the fuel properties of biodiesel from *Isochrysis* and may provide a path
14 toward improved biodiesel fuels from other algal species.

15 **Introduction**

16 Following an approximately ten year hiatus after the United States Department of Energy's
17 Aquatic Species Program (U.S DOE ASP) ended in 1996 [1], there has been a great resurgence
18 of interest in algae as a potential source of biofuel in the last decade [2, 3, 4]. Some species of
19 algae have reportedly very high oil contents (15-300 times more oil for biodiesel production than
20 traditional crops on an area basis) [5-7], can be grown on brackish- or wastewater and otherwise
21 non-cultivable land [8], and may achieve higher CO₂ sequestration capabilities than terrestrial
22 plants [9-11]. Another suggested benefit described by both critics [12] and proponents of algal
23 biofuel programs and within the United States Department of Energy's National Algal Biofuels

1 Technology Roadmap [13], is the potential for other high-value co-products that may be used to
2 offset production costs of the fuel.

3 However, the properties of the fuel produced must also be considered because it has been
4 discussed that many algal biodiesel fuels may have problematic properties [14]. In order to be
5 commercialized, biodiesel must meet a variety of specifications described in the standards
6 ASTM D6751 and EN 14214 in the U.S and Europe, respectively. Requirements include
7 minimum values for oxidative stability and cetane number, allowable ranges for kinematic
8 viscosity, maximum free and total glycerol and heteroatom (Na, K, S, P, Ca, Mg) contents, while
9 cold-flow properties are addressed by a report of the cloud point (CP) in ASTM D6751 due to
10 different requirements dictated by time of year and geographic location. Others have noted the
11 challenges associated with examining the fuel properties of algal biodiesel due to the small
12 experimental quantities that are generally produced compared to the larger amounts required for
13 each individual test [15]. For this reason, many of the reports describing algal biodiesel fuel
14 properties are incomplete with algal biodiesel blended with petrodiesel to complete the fuel
15 testing [16] or have relied on predictions [17] and simulations such as using a mixture of
16 biodiesel from vegetable oil and fish oil to model algal biodiesel fuel properties [18].

17 Our group has been investigating biodiesel and other co-products from the marine microalgae
18 *Isochrysis* sp. [19-21]. *Isochrysis* is one of only a few species of algae currently grown
19 industrially, harvested for purposes of mariculture, and available internationally on multi-
20 kilogram scale from several suppliers [22]. Crude *Isochrysis* biodiesel, prepared from the total
21 lipid extract of dried *Isochrysis* biomass, suffers from severe cold-flow issues (i.e. it is solid at
22 room temperature) [19]. We showed that these cold-flow problems are likely associated with
23 contamination of this material by a unique suite of lipids known as polyunsaturated long-chain

1 alkenones biosynthesized by a few haptophyte algae including *Isochrysis* (Figure 1) [23-25].
2 Owing to their long carbon-chain length (36-40 carbons), non-methylene interrupted *trans*-
3 double bonds, and resulting high melting points, we demonstrated that even minor amounts of
4 alkenones can have a deleterious impact on the CP of a B20 biodiesel blend [19].

5 We therefore developed a protocol for preparing an “alkenone-free” *Isochrysis* biodiesel based
6 on saponification/extraction techniques, and sufficient quantities were produced to allow for a
7 range of fuel tests according to ASTM standards [20]. Unfortunately, however, we were unable
8 to measure a CP due to the dark color of this material and thus confirm our original hypothesis
9 connecting alkenones to the obvious poor cold-flow properties of our crude biodiesel. Herein we
10 report the production and comprehensive fuel testing of a decolorized alkenone-free *Isochrysis*
11 biodiesel, with pigments removed using montmorillonite K10 clay. In addition to allowing for
12 CP analysis, a comparison of other fuel property values for our decolorized vs. non-decolorized
13 biodiesel fuels revealed important insights about the potential impact of common pigments on
14 fuel properties, along with remaining challenges for the production of a biodiesel from *Isochrysis*
15 meeting ASTM standards as well as from presumably other algal feedstocks.

16 **Materials and Methods**

17 **Microalgae and sample preparation.** The marine microalgae *Isochrysis* sp. “T-iso” used in
18 the present study was obtained as strain CCMP1324 from Reed Mariculture (San Jose, CA) [27].
19 Approximately eight kilograms of wet biomass (20% biomass w/w) was freeze-dried in ~100 g
20 batches, which resulted in an *Isochrysis* sp. as a greenish, dark-brown solid with an earthy
21 seaweed-like smell. The resulting dry *Isochrysis* was then processed batchwise (100-200 g dry
22 biomass) into biodiesel samples (e.g. D-*Iso*-1, D-*Iso*-2, and D-*Iso*-3) that were analyzed
23 separately (*vide infra*).
24

1 **Extraction and processing of lipids to produce an alkenone-free biodiesel.** The dry
2 *Isochrysis* sp. biomass was extracted and the obtained lipids processed into isolated alkenones
3 and biodiesel as previously described [19-21]. Briefly, dry biomass is extracted with hexanes in a
4 Soxhlet extraction apparatus. Hexanes were removed with a rotary evaporator giving a dark
5 green near-black solid. The hexanes-extractable material (“hexane algal oil”) is then saponified
6 (KOH, MeOH, CHCl₃) allowing for separation of the resulting free fatty acids (FFAs) and
7 alkenone-containing neutral lipids. Acid-catalyzed esterification (MeOH, cat. H₂SO₄) of the
8 FFAs gives alkenone-free “non-decolorized” biodiesel (i.e. fatty acid methyl esters, FAMES).

9 **Biodiesel decolorization [26].** To the dark green colored biodiesel obtained above (15 g) at
10 60 °C was added montmorillonite K 10 (MK10) powder (3.0 g, 20% w/w of the biodiesel) and
11 the mixture was stirred for 1 hr. The solution was then filtered through celite with hexanes and
12 the hexanes were removed on a rotary evaporator to produce an orange/red biodiesel (13 g, on
13 average 90% w/w mass recovery). Samples were stored at 4 °C during which time some settling
14 of insoluble material (<10% w/w) occurred. Decanting gave a clear homogeneous biodiesel that
15 was analyzed separately.

16 **Analysis by gas chromatography with flame ionization detection (GC-FID).** Fatty acid
17 profiles were determined by gas chromatography utilizing a Perkin-Elmer Clarus 580 gas
18 chromatograph equipped with a DB-88 [(88 % cyanopropyl) methylarylpolysiloxane] column
19 (30 m x 0.25 mm ID x 0.20 µm film thickness) and otherwise as described in the literature [27].
20 Common fatty acid methyl esters were verified by retention time comparison with authentic
21 samples obtained from Nu-Chek Prep, Inc. (Elysian, MN). Additionally, gas chromatography-
22 mass spectrometry (GC-MS; Agilent Technologies 6890N gas chromatograph coupled to an
23 Agilent Technologies 5973 mass selective detector) was performed under identical conditions of

1 temperature program and column to analyze components such as C18:4 for which no authentic
2 standards are available with the results applied to GC quantitation.

3 **Fuel Properties.** Cetane numbers were determined as derived cetane number (DCN) using an
4 Ignition Quality TesterTM (IQTTM) as described in ASTM D6890 at Southwest Research
5 Institute, San Antonio, TX [28]. CP was determined with a Phase Technology (Richmond, BC,
6 Canada) cloud, pour and freeze point analyzer. Kinematic viscosity was determined according to
7 the procedure described in the standard ASTM D445, oxidative stability according to EN 14112
8 using a so-called Rancimat instrument, and lubricity with a high-frequency reciprocating rig
9 (HFRR) lubricity tester according to ASTM D6079. Density was measured with an Anton Paar
10 (Anton Paar USA, Richmond, VA) DMA 4500 density meter. Free and total glycerol as well as
11 monoglyceride content were determined according to the gas chromatographic procedure
12 described in the standard ASTM D6584.

13 **Results and Discussion**

14
15 **Table 1** contains information on the fatty acid methyl ester composition of the decolorized
16 biodiesel. **Table 2** lists the fuel properties of this material.

17 **Production of a purified non-decolorized *Isochrysis* biodiesel.** Results for the extraction and
18 subsequent separation of FFAs and neutral lipids from dry *Isochrysis* biomass, followed by
19 esterification of the FFAs to biodiesel were consistent with those previously reported [19-21].
20 The hexane algal oil obtained was a glossy, dark green/near-black grease-like material (typically
21 20% w/w of the dry biomass). Saponification of hexane algal oil with KOH in methanol then
22 allowed for separation of the saponified FFAs from alkenones and other non-polar compounds
23 with yields consistently quantitative (60% FFAs + 40% neutral lipids). Acid-catalyzed

1 esterification of the FFAs produced a dark green colored biodiesel (“non-decolorized biodiesel”)
2 that has previously undergone extensive analysis (see “Non-D”, **Table 2**) [20].

3 **Decolorization of the biodiesel using montmorillonite K 10.** Based on the work of Dalai
4 [26], stirring our green biodiesel over 20% (w/w) MK10 for 1 h at 60 °C proved highly effective
5 in removing pigments, visualized by the color change of the biodiesel and the MK10 itself going
6 from a white powder to black (Figure 2). After decolorization, a small amount (<10% w/w) of
7 “grainy material” previously described became evident [21]. Upon storage these compounds
8 settled to the bottom of the vessel and could be easily removed by decanting or, if necessary,
9 centrifugation. Mass recoveries of particulate-free biodiesel after decolorization were generally
10 85-95% (w/w).

11 **FAME analysis of decolorized *Isochrysis* biodiesel.** The FAME profiles of the non-
12 decolorized biodiesel and subsequently decolorized biodiesel were nearly identical as an
13 indication of a successful decolorization process (**Table 1**) [20]. For each, the major fatty acid
14 was 18:4, at approximately 21 mg/g of crude FAME. Polyunsaturated fatty acid (PuFAMES,
15 more than two double bonds) content was highest for the non-decolorized biodiesel (Non-D,
16 42.4%) but in the same range for all samples (e.g. 39.7% = 10.3 (18:3) + 21.0 (18:4) + 8.4 (22:6)
17 for D-*Iso*-1). Docosahexaenoic acid (DHA; 22:6), was also present to a large extent (6.9-8.4
18 mg/g of decolorized FAME). Other individual FAMES for the different batches are listed in
19 **Table 1**. The sample produced from a hexane algal oil that had been isolated and stored at 20 °C
20 for approximately two years (D-*Iso*-2) had a slightly higher proportion of saturated FAMES
21 (30.6% vs. 26.5 % for D-*Iso*-3) and lower unsaturated FAME content, consistent with stability
22 trends for these compounds. For all of the samples, at least 95% of the material could be
23 identified as a FAME. It may be noted that the present *Isochrysis*-derived biodiesel would not

1 meet the European biodiesel standard EN 14214 concerning its specification < 1% fatty acids
2 with more than three double bonds but the American standard ASTM D6751 does not have this
3 restriction, so that the addition of antioxidants may overcome this issue for the North American
4 market.

5 **Pigment removal and cloud point analysis of an *Isochrysis* biodiesel.** Cloud point has been
6 shown to be the most stringent specification regarding cold flow properties but can be correlated
7 with other tests such as the cold filter plugging point [29]. Experimental CP data for a biodiesel
8 from an algal feedstock are quite scarce with exceptions being [30, 31]. For instance, a CP value
9 is absent from otherwise fairly extensive testing of biodiesel from the microalga *Schizochytrium*
10 *limacinum* [32]. While these authors do not state the reason for this omission, the overall
11 unavailability of this data may be due to the same difficulties we encountered with our initial
12 *Isochrysis*-derived product being too dark in color to measure a CP.

13 Like other land and aquatic photosynthetic organisms, *Isochrysis* contains chlorophylls and
14 pheophytins that can degrade into compounds like pheophorbides [33]. It was reported that
15 chlorophyll and its derivatives have a negative effect on the stability of vegetable oils [34].
16 Various solid materials such as clays and activated carbon can be used to selectively remove
17 chlorophylls and pheophytins from these mixtures [35]. For instance, Issariyakul and Dalai
18 demonstrated the effectiveness of montmorillonite K 10 (MK10) clay for decolorizing greenseed
19 canola oil in connection with biodiesel production [26]. Applied to our green non-decolorized
20 *Isochrysis* biodiesel, we found that stirring over 20% (w/w) MK10 at 60 °C for 1 hr resulted in a
21 dramatic reduction in pigment content by visual inspection (*ref.* Figure 2). Moreover, absorbance
22 peaks corresponding to chlorophylls and pheophytins were no longer observed in the now
23 orange/red biodiesel [See Supplementary Material]. Presumably it is the Lewis basic porphyrin

1 structures within these compounds interacting strongly with the acidic MK10 clay that accounts
2 for the selectivity of this process [36], giving on average 90% mass recoveries for now
3 decolorized biodiesel.

4 The measured CP values for our decolorized biodiesel samples were as expected low, however
5 the values obtained (-6.0 and -6.0, duplicate analysis for D-*Iso*-1; -5.8 and -5.6 °C duplicate
6 analysis for D-*Iso*-2) were lower than what would be predicted based on the FAME profile [37].
7 The pour points (PP) were also exceptionally low (-8.6 and -8.4, duplicate analysis for D-*Iso*-1; -
8 6.0 and -6.0 °C, duplicate analysis for D-*Iso*-2) given the fairly large amounts of saturated
9 FAMEs (e.g. ~15% C16:0) in the material. For comparison, soybean biodiesel (SME) contains
10 roughly 10% methyl palmitate (C16:0) and has CP and PP values of 1 °C and 0 °C respectively
11 [38]. SME, however, also contains approximately 5% of even higher melting methyl stearate
12 (C18:0) of which there are only traces in *Isochrysis*. CP is sensitive to minor amounts of higher
13 melting components [19, 39] so that the different methyl stearate contents may at least partially
14 explain the large disparity in CP between SME and our decolorized *Isochrysis* biodiesel. Efforts
15 are ongoing to better understand the cold-flow properties of our *Isochrysis* biodiesel toward
16 further refined predictive CP models.

17 **Cetane number (CN).** The ASTM D6751 and EN 14214 standards prescribe minimum CN
18 values of 47 and 51 respectively for commercial biodiesel. For our non-decolorized biodiesel, we
19 had previously measured a CN of 36.5 (Table 2) [20]. After decolorization, both samples
20 exhibited a higher CN and with one meeting the ASTM standard (42.3 and 48.4). Care must be
21 taken, however, when calculating and comparing CN values as these numbers are not absolute
22 and the results often variable [40]. In a recent comprehensive evaluation of this parameter for
23 biodiesel, the CN of methyl oleate was at best estimated to be in the range of 56-58 using data

1 from 23 different measurements [40]. Nonetheless, pigment removal improved the CN. While
2 too much emphasis cannot be placed on the actual value for reasons stated above, the CN of our
3 decolorized *Isochrysis* biodiesel is likely close to the ASTM standard (avg. for samples = 45).

4 **Kinematic Viscosity.** Algal biodiesel fuels with high PUFAME content would be expected to
5 have lower kinematic viscosity than vegetable oil-derived biodiesel, although some higher
6 viscosities have also been reported for simulated algal FAME mixtures [41]. Previously we
7 reported a kinematic viscosity of 2.46 mm²/s at 40 °C for our non-decolorized biodiesel [20],
8 which was lower than the 3.2 mm²/s that would be estimated based on the FAME profile [20].
9 Attempts to check the accuracy of this result failed, however, as the samples consistently
10 plugged viscometer tubes due to the presence of some insoluble material. Our final decolorized
11 biodiesel product was free of these particulates and the kinematic viscosity measured (3.38 and
12 3.76 mm²/s) was similar to the predicted value (3.2 mm²/s). The measured kinematic viscosities
13 are on the low end of the range prescribed ASTM D6751 standards and close to the minimum
14 specified in EN 14214. However the technical justification for the higher minimum viscosity
15 specification of 3.5 mm²/s in EN 14214 is not clear as conventional petrodiesel fuels generally
16 exhibit viscosity values below 3.5 mm²/s.

17 **Oxidative Stability.** All non-decolorized and decolorized *Isochrysis* biodiesel fuels exhibited
18 poor oxidative stability, well below the ASTM minimum specification. This is due to the high
19 PuFAME contents of these fuels (approximately 40%, *ref. Table 1*) as increased unsaturation
20 within a fatty acid carbon-chain results in decreasing oxidative stability [42]. The highest
21 oxidative stability among the samples tested might therefore be expected for D-*Iso*-2 with a
22 higher proportion of saturated FAMES and lack of pigments that have been reported to adversely
23 affect biodiesel oxidative stability [26]. The oxidative stability of D-*Iso*-2 was in fact lower than

1 for the decolorized D-*Iso*-1 (0.05 h vs. 0.35 h) and essentially the same as the non-decolorized
2 biodiesel (0.06 h). However, the oxidative stability of all samples is very low so that the
3 differences would likely not have any major effect under practical aspects. Furthermore, the
4 percentage of identified FAMES was lowest for the decolorized D-*Iso*-2 (88.5%). Minor
5 components of undetermined nature within this sample may affect the oxidative stability more
6 than their minor amounts would indicate.

7 **Lubricity and Density.** Lubricity for diesel fuels is becoming an increasingly recognized
8 property with more stringent limitations on sulfur content for environmental reasons. The HFRR
9 wear scars of the decolorized *Isochrysis* biodiesel samples were well below the maximum wear
10 scars of 460 μm and 520 μm prescribed in the standards EN 590 and ASTM D975, respectively.
11 These values (131 and 125 μm , duplicate analysis for D-*Iso*-1; 136 and 133 μm , duplicate
12 analysis for D-*Iso*-2) represent an improvement from the non-decolorized biodiesel (260 μm),
13 and are now more in line with measured lubricities for biodiesel from other feedstocks (e.g. soy-
14 derived biodiesel around 130 μm) [43].

15 Fuel density relates to fuel performance (e.g. within the injection system). Differences in
16 density between biodiesel and petrodiesel creates some concern about potential mismatching of
17 engine parameters when using this type of fuel in engines optimized for petrodiesel [44]. For
18 this reason, the European standard EN-590 establishes a density range for diesel fuels of 820 -
19 845 kg/m^3 at 15 °C. Biodiesel tends to have a higher density than petrodiesel [45], and accurate
20 density data are needed to calculate appropriate blend ratios that will meet this specification.

21 The availability of experimental density data for algal biodiesel fuels is limited [16, 46,47], but
22 can be predicted from FAME profiles using linear mixing rules and the known densities of neat
23 FAMES [17]. The measured density for our non-decolorized *Isochrysis* biodiesel at 15 °C was

1 934.92 kg/m³, higher than the maximum (900 kg/m³) prescribed in EN 14214 [24]. Post-
2 decolorization, the densities measured were 895.5 and 898.5 kg/m³ for D-*Iso*-1 and D-*Iso*-2
3 respectively, which now just fall within the EN 14214 range (max. = 900 kg/m³). Because the
4 FAME profiles and contents of the non-decolorized and decolorized biodiesel fuels were very
5 similar (*ref.* **Table 1**), the data suggests that the presence of pigments results in a higher density.
6 However, the extent of their impact has yet to be rigorously investigated and it remains to be
7 determined if such low levels of these compounds (max. = 5% w/w) could account for the
8 observed differences of our decolorized and non-decolorized biodiesel fuels.

9 **Glycerol and FFA and moisture Content.** Our processing consistently produces a biodiesel
10 that meets the free and total glycerol amounts as well as moisture content (345 ppm for D-*Iso*-1)
11 according to ASTM D6751 and EN-14214. The acid values, however, exceeded the limitations
12 according to these specifications. Problems associated with acidity for diesel fuels center on the
13 possibility of corrosion and potential formation of engine deposits. For biodiesel, the acid value
14 indicates FFA content [48]. Because our processing involves first converting all acylglycerols to
15 FFAs for the purpose of separating alkenones and other neutral lipids, the acid value essentially
16 represents the percent yield for our esterification step, or 98.6% (Acid Value = 3.029 = 1.383%
17 FFAs) and 97.7% (Acid Value = 5.139 = 2.347% FFAs) for D-*Iso*-1 and D-*Iso*-2, respectively.
18 The ASTM D6751 and EN-14214 acid value limit of 0.50 corresponds to an exceedingly low FA
19 content of approximately 0.25%, meaning we would need to achieve an esterification yield of
20 99.75% or reduce the acid value of the final product by other means [49]. Future work will
21 therefore include an optimization of this parameter toward the production of a commercially
22 viable *Isochrysis* biodiesel.

23

1 **Conclusion**

2 Pigments such as chlorophylls and other derivatives can be efficiently removed from
3 *Isochrysis* biodiesel using montmorillonite K10 clay. The process was performed on sufficient
4 scale to allow for comprehensive fuel testing of the resulting decolorized biodiesel. This was
5 made possible in part due to the commercial availability of *Isochrysis* in multi-kilogram
6 quantities from several suppliers. Results from the fuel tests provided important experimental
7 data that can be used to validate and refine often used predictive models for algal biodiesel fuel
8 properties. For instance, oxidative stability remains an issue for our *Isochrysis* (and presumably
9 other algal) biodiesel, and appears highly sensitive to even minor amounts of PUFAMEs.
10 Through the decolorization process we were now able to obtain a CP, with the measured CP for
11 our decolorized biodiesel lower than what would be predicted based on the FAME profile. A
12 comparison of the fuel testing results for our decolorized sample to that previously obtained for a
13 non-decolorized *Isochrysis* biodiesel also revealed certain impacts of pigments on fuel
14 properties. Specifically, pigment removal resulted in a 24% increase in CN (from 36.5 to 45.4
15 (avg.)), 40% increase in kinematic viscosity (from 2.5 to 3.5 mm²/s), a 50% decrease in lubricity
16 (from 260 to 131 μm), and 4% decrease in density (from 935 to 897 kg/m³). There remains,
17 however, approximately 5% unaccounted for material in the samples tested making any absolute
18 claims about the role of pigments on fuel properties difficult. Work is therefore ongoing to fully
19 characterize these mixtures, along with continued studies toward the production of an ASTM-
20 certified *Isochrysis* biodiesel.

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3 **Supplementary Material.** ¹H NMR and UV absorbance spectra of the decolorized *Isochrysis*
4 biodiesel.

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1 **Table 1.** FAME Composition of Non-Decolorized (Non-D),²³ and Decolorized *Isochrysis*
 2 Biodiesel (D-*Iso*) Samples.

FAMES	Non-D ^A	D- <i>Iso</i> -1	D- <i>Iso</i> -2 ^D	D- <i>Iso</i> -3
14:0	13.9	14.3	17.1	16.4
15:0	0.2	ND	0.4	trace
16:0	11.0	10.1	12.4	10.1
16:1 Δ9	5.9	7.0	6.6	7.6
16:2	0.6	ND ^F	trace	ND
16:3	0.7	ND	ND	ND
18:0	Trace	ND	1.1	Trace
18:1	11.4 ^B	10.8 ^B	11.0 ^B	12.1
18:2	7.4	9.9	6.5	8.1
18:3	6.8 ^C	10.3 ^C	6.9 ^C	8.5
18:4	20.7	21.0	18.3	19.8
18:5	1.6	ND	ND	ND
20:5	1.6	ND	ND	ND
22:5	1.1	ND	ND	ND
22:6	10.6	8.4	8.2	6.9
Σ	94.9	91.8^E	88.5^E	89.8^E

3 ^AAverage values from three separate samples. ^BCombined 18:1 Δ9 + 18:1 Δ11. ^CCombined
 4 Δ6,9,12 and Δ9,12,15 isomers. ^DPrepared from an older hexane algal oil that was stored at ~20
 5 °C for two years before being used for this study. ^EThe remaining material is roughly 50:50 other
 6 FAMES and non-FAME components (Total ~95% FAME). ^FND = Not detected.

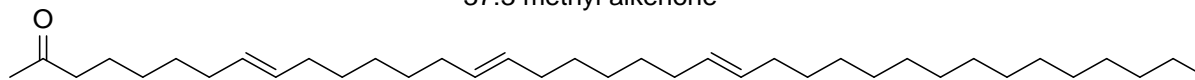
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1 **Table 2.** Fuel Properties of Non-decolorized (Non-D)²³ and Decolorized *Isochrysis* (D-Iso)
 2 Biodiesel Fuels.

Property	Non-D	D-Iso-1	D-Iso-2 ^d	ASTM D6751	EN 14214
Cetane number	36.53	42.3	48.4	47 min	51 min
Kinematic viscosity (40°C, mm ² /s) ^a	2.46	3.38	3.76	1.9-6.0	3.5-5.0
Oxidative stability (110°C; h)	0.06	0.35	0.05	3 min	6 min
Cloud Point (°C)	ND	-6.0, -6.0 ^c	-5.8, -5.6 ^c	Report	
Pour Point (°C)	ND	-8.6, -8.4 ^c	-6.0, -6.0 ^c	Report	
Density (15°C, kg/m ³)	934.92	895.52	898.54	---	860-900
Lubricity (µm; 60°C; HFRR)	260	131, 125 ^c	136, 133 ^c	520 max (ASTM D975)	460 max (EN 590)
Free glycerol (mass %)	0	0.004	0.0045	0.020 max	0.02 max
Total glycerol (mass %)	0.029	0.025	0.033	0.240 max	0.25 max
Monoglycerides (mass %)	0.034	0.029	0.020	0.40 max	0.70 max
Acid Value (mg KOH·g ⁻¹)	ND	3.029	5.139	0.50 max	0.50 max
Moisture	ND	345 ppm	ND	0.05% max (v/v)	500 max mg/kg

3 ^aDetermined on an individual batch (~10 g) prior to blending. ^bLimits listed as given in the
 4 standards. ^cDuplicate measurements. ^dPrepared from an older algal oil, stored at ~20 °C for two
 5 years (*ref.* Table 1). ^eND = Not determined.
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 8

37:3 methyl alkenone



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2 **Figure 1.** Structure of a common alkenone produced by *Isochrysis* sp. Nomenclature is # of
3 carbons:# of double bonds, where methyl refers to a methyl ketone.

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5
6 **Figure 2.** Comparison of non-decolorized (left) and decolorized (right) *Isochrysis* biodiesel
7 fuels.