



Fuel property evaluation of unique fatty acid methyl esters containing β -hydroxy esters from engineered microorganisms

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ABSTRACT

Unique fatty acid methyl esters (FAME) containing β -hydroxy esters were produced using an engineered microorganism by glucose fermentation. This study investigated the properties of the unique FAME mixture both neat and in blends with conventional diesel, as well as properties of β -hydroxy esters. The unique FAME blend contained relatively shorter-chain FAME (average fatty acid chain carbon number 14.6) with 58 % mono-unsaturated fatty acids and 9 % saturated and monounsaturated β -hydroxy acid chains. The unique FAME had significantly lower distillation T90 (321 °C versus 352 °C) and higher cetane number (56.7 versus 52) compared to soy biodiesel. Cloud points were within method repeatability. Unexpectedly (because of the lack of methylene-interrupted double bonds), the unique FAME had low oxidation stability (1.5 h) as determined by Rancimat induction period. Stability could be improved through addition of commonly used antioxidants. We speculate that monounsaturated β -hydroxy FAME may be the source of this instability. Blends with conventional diesel up to 50 vol% showed similar kinematic viscosity (within method repeatability) as blends of conventional FAME. The unique FAME had no effect on distillation T90 even at the 80 % blend level. A 30 vol% blend into conventional diesel had a Rancimat induction period of only 2 h, very nearly the same as the neat unique FAME sample. The addition of antioxidants produced blends of acceptable stability. Based on an assessment of the properties of individual β -hydroxy FAME molecules, they have higher boiling point, higher cloud point, lower cetane number, and potentially lower storage stability than analogous FAME not having the β -hydroxy group. Removing them from the fuel product in the production process may result in a biodiesel product with superior properties to what is on the market today.

1. Introduction

The transportation sector is a major energy consumer and a contributor of CO₂ and greenhouse gas emissions [1]. Electrification of large heavy-duty vehicles can be challenging because of the required energy density, weight, and size of the batteries. This potentially makes lower-carbon liquid fuels a viable candidate for decarbonization of heavy-duty transport, especially considering compatibility with current infrastructure [2]. Currently, first-generation biofuels provide 4.2 % of transport sector energy, represented primarily by ethanol and biodiesel/renewable diesel [3]. Biodiesel is typically produced via the transesterification of triglyceride feedstock such as vegetable oils or

animal fats to produce fatty acid methyl esters (FAME) in the presence of sodium alkoxide catalyst. Alternatively, hydroprocessing of the same feedstocks results in paraffinic hydrocarbon products, known as renewable diesel [4]. The use of biodiesel/renewable diesel can reduce life cycle greenhouse gas emissions 40 %–86 % relative to petroleum diesel, depending on feedstock and assumptions about land use change [5]. The properties, economics, and sustainability profile make biodiesel a desirable fuel, but the supply of fats and oils feedstocks is limited [6]. This has led to research into advanced biofuels produced from other bio-derived feedstocks (e.g., algae, municipal wastes, lignocellulosic biomass [7–11]).

There has also been research using engineered microorganisms

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[12–14] to produce FAME from renewable biomass. Lu and coworkers engineered *Eschericia coli* to produce diesel boiling range free fatty acids (C12 to C18) from glucose [12]. Later, Steen and colleagues reported on engineering of *E. coli* to produce diesel boiling range fatty acid ethyl esters directly from glucose and endogenous ethanol [13]. Nawabi and colleagues also engineered *E. coli* to produce fatty acids and a fatty acid methyl transferase that catalyzed formation of FAME with exogenous methanol [14]. These researchers also identified a thioesterase enzyme that caused the production of β -hydroxy fatty acids (Fig. 1) that were also converted to FAME. Previously, Zheng and coworkers had shown that thioesterase II was instrumental in formation of β -hydroxy decanoic acid as a component of a metabolic pathway to formation of poly-hydroxyalkanoates [15]. Reducing this line of research to practice, Pandey and coworkers describe in a patent application a process for production of FAME with an average carbon number of 14.5 and containing around 10 % β -hydroxy FAME. Conditions were optimized to avoid glucose accumulation and reduce common byproducts of *E. coli* fermentation, such as formate and acetate. Fatty esters are then recovered and separated from the fermentation products [16]. This paper describes the composition and properties of this β -hydroxy FAME containing product and for the first time, the properties of diesel-boiling-range β -hydroxy FAME.

Previous research on fuel properties has focused on gasoline-boiling-range β -hydroxy esters as potential biofuels. Zhang et al. [17] and Wang et al. [18] investigated the acid-catalyzed hydrolysis of poly-hydroxyalkanoates to produce high-purity hydroxybutyrate methyl esters, as well as longer-chain esters. They measured the heat of combustion of pure esters and blended with different alcohols, diesel, and gasoline. Generally, blending of these esters lowered the heat of combustion of the parent fuels, except for blends with ethanol [17]. Hydroxybutyrate methyl esters were tested as an additive to gasoline and showed better properties compared to gasoline–ethanol blends. The impact on octane number was minor [18].

This study investigates the properties of a unique FAME mixture containing β -hydroxy esters both neat and in blends with conventional diesel, as well as properties of individual β -hydroxy ester molecules. The objective of this research was to determine if the presence of β -hydroxy esters imparted improved properties and the extent to which this FAME mixture was suitable for use in diesel engines. The approach taken was a detailed assessment of fuel chemistry and properties for the FAME mixture, its blends with conventional diesel fuel, and for a set of pure β -hydroxy esters.

2. Methods

2.1. Fuel samples

The FAME samples investigated in this study were provided by Genomatica, San Diego, CA, USA. They invented a novel engineered

E. coli to ferment glucose in the presence of methanol. Fatty esters are then recovered and separated from the fermentation products [18].

Three samples were studied in this work:

1. A mixture of saturated and unsaturated β -hydroxy FAME (C14 and C16) and conventional FAME (C12–C16) representing the actual fuel that would be produced by this process.
2. 95 % purity β -hydroxy FAME methyl-3-hydroxytetradecanodate (C14:0).
3. 98 % purity mixture of saturated and unsaturated (38 % C14:0 + 60 % C14:1) β -hydroxy FAME.

These samples are referred to as Sample 1, Sample 2, and Sample 3, respectively. Sample 1, also referred to as unique FAME, was blended with conventional petroleum diesel (Diesel A) to study the effect of β -hydroxy FAME in fuel blends. In some cases, a conventional diesel fuel that had been clay treated (Diesel B) to remove all fuel additives (e.g., corrosion inhibitors, lubricity enhancers, conductivity enhancers, antioxidants) was used. Soy biodiesel was acquired from a commercial producer and used for comparison purposes. Relevant properties of these blendstocks are reported in the following sections or shown in the Supporting Information (SI). Methyl-3-hydroxy hexanoate was obtained from MilliporeSigma, St. Louis, MO, USA. Small samples of additional saturated methyl β -hydroxy esters of varying chain length were obtained from Matreya, LLC, State College, PA, USA.

2.2. Chemical analysis and physical property measurement

The composition of the three samples was obtained using a gas chromatography method described in the SI. Other analyses included (test method employed in parenthesis) total acid number (TAN) (D664), sulfated ash (D874), water and sediment (D2709), glycerin and glycerides content (D6584), phosphorus (D4951), and trace metals (D7111 Mod). Physical properties were measured following ASTM methods to determine cloud point (D5773), flash point (D6450), distillation temperature T90 of neat FAME (D1160), distillation of diesel fuels and FAME blends (D86 or D2887 as noted in text), indicated cetane number (ICN) (D8183), density (D4052), kinematic viscosity at 40 °C (D445), copper corrosion (D130), lubricity (D6079), and net heating value (D240). Detailed citations to all standard methods are provided in the SI. In some cases, cetane number (CN) was estimated using the National Renewable Energy Laboratory's cetane number prediction tool [19], which uses molecular structures as simplified molecular-input line-entry system (SMILES) strings as input. Differential scanning calorimetry (DSC) was used to measure melting point, and thermogravimetric analysis (TGA) at ambient pressure with nitrogen purge gas was used to measure boiling point [20] of pure compounds in some cases. A heat/cool/heat cycle run at a 10 °C/min temperature ramp was utilized to determine the melting point by DSC.

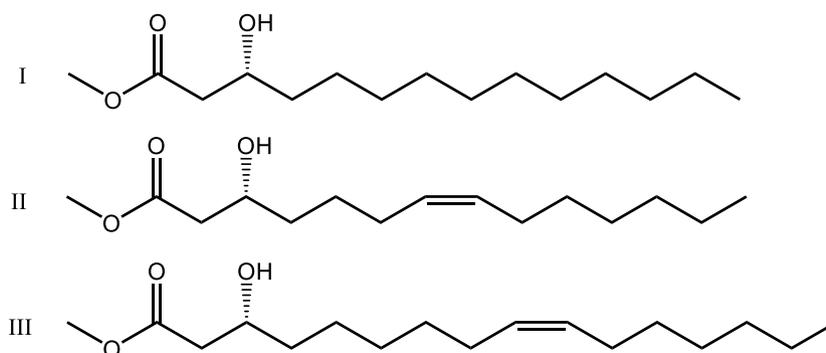


Fig. 1. Examples of diesel-boiling-range β -hydroxy methyl esters. Groups around the beta-carbon atom are arranged in the R-configuration.

Yield sooting index (YSI) was measured by Princeton University using the method developed by McEnally and Pfefferle [21] or predicted using the National Renewable Energy Laboratory's YSI prediction tool [22]. Higher YSI is correlated with higher soot formation tendency. YSI was measured in an atmospheric pressure, co-flow diffusion flame. The main fuel stream in the center tube (1.2 cm in diameter) was a methane-N₂ mixture loaded with 1000 ppm of the fuel being tested. The liquid fuel was pre-vaporized by a syringe pump and a vaporizer system. The two concentric co-flows (1-mm gap) were air and the sheath flow (N₂). The purpose of the outer tube was to isolate the flame from the lab environment. In this study the index compounds were reagent grade hexane and benzene, assigned respective YSI values of 30 and 100. Due to the wide range of YSI for the tested fuels, reagent grade toluene was used as validation fuel so that all samples in this study were covered in the test range. The flow rate of each doped fuel was calculated based on its properties. The whole system was preheated to make sure that the fuel fully evaporated before reaching the burner. A methane flame (no doping) image was used as reference for calibration before each experiment. After the doped fuel flame stabilized, an intensified charge-coupled device camera was used to take images; 300 images were recorded in each test, and at least three repeated tests were done for each fuel. MATLAB (Version R2022a, Natick, Massachusetts: The MathWorks Inc.) was used in data processing and error analysis of the YSI results.

Oxidation stability was investigated using EN15751 (Rancimat induction period) an accelerated test used in fuel quality specifications. Oxidation stability was also evaluated using ASTM D4625, a long-term storage test. D4625 involves storing a 400-mL sample at 43 °C in a container open to ambient air for many weeks. In the standard test, the sample is then filtered to measure insoluble formation. We modify the test method to remove aliquots periodically for measurement of peroxides [23,31]. The unique FAME and blends with diesel fuel were blended with the antioxidants tert-butyl hydroquinone (TBHQ, Millipore Sigma, 97 % purity) or butylated hydroxytoluene (BHT, Innospec, 99 % purity) for some experiments. TBHQ and BHT are commonly used to stabilize B100 and biodiesel blends [24,25].

3. Results and discussion

3.1. Chemical analysis of FAME mixture

Although diesel-boiling-range β -hydroxy FAME has not previously been investigated in terms of fuel properties, a great deal is known about how molecular structure impacts the properties of conventional FAME biodiesel. The properties of biodiesel are determined by the feedstock fatty acid structure, specifically the chain length and degree of unsaturation [4]. Hoekman et al. [26] report the composition and properties of 12 common biodiesels produced from vegetable oils. They found that the CN, oxidation stability, kinematic viscosity, and cold flow properties were strongly correlated to the degree of unsaturation of the FAME. A high level of polyunsaturated fatty acid chains can lead to poor oxidation stability, requiring treatment with antioxidant additives—a strategy commonly used in the market today. Unsaturated fatty acid chains have also been correlated with higher NO_x emissions [27], likely because of changes in combustion stoichiometry at ignition and in the standing autoignition zone near the flame lift-off length [28] that leads to higher peak combustion temperature. Unsaturated chains have low melting points, while high levels of saturated FAME can result in high cloud point and consequent low-temperature operability challenges [26]. At the same time, saturated fatty acids have high CN. Long-chain monounsaturated fatty acid chains have been identified as a good compromise between high CN, low NO_x emissions, and low melting temperature [29]. Biodiesel from the feedstocks used today consists primarily of C16 and C18 fatty acid chains and consequently has a high boiling point, above the 338 °C T90 limit for diesel fuel in ASTM D975. While this has not been problematic for blending at current levels (up to

20 vol%), the high boiling point may limit the potential for very high blends or use of neat biodiesel needed for full decarbonization. Moreover, the presence of impurities (sterol glucosides or saturated mono-glycerides) also affects the FAME properties, as these can improve lubricity [30] but also precipitate, causing cold temperature operability problems [31,32]. Given this background, we assess the properties of a unique FAME sample containing β -hydroxy esters, as well as the properties of β -hydroxy esters in neat form.

The analysis for the unique FAME sample (Sample 1) is detailed in Table 1. This sample is a mixture of 86 % FAME and 9.2 % β -hydroxy FAME. The composition is dominated by palmitoleic (C16:1, 39.8 %), myristic (C14:0, 19.5 %), and palmitic (C16:0, 15 %) fatty acids, which are relatively shorter-chain fatty acids compared to those in rapeseed, tallow, and soy biodiesel [26], which are dominated by C18 fatty acid chains (Fig. 2). The average carbon number of the fatty acid chains is 3 less for Sample 1 compared to soy biodiesel. The unique FAME contains only saturated (37.4 %) and monounsaturated (55.9 %) fatty acid chains. This contrasts with soy biodiesel, which contains 15 % saturated with 57 % di- and tri-unsaturated chains. Further analysis for the unique FAME is shown in Table 2, where ash and metals are very low and only low traces of glycerin and glycerides were detected due to the unique production method compared to the typical transesterification for biodiesel production. Free and total glycerin values are well below the limits in the ASTM D6751 specification for B100 biodiesel. Sample 1 is 95.2 % FAME and β -hydroxy FAME, with the balance unidentified. A priority for future research is to determine what other materials are present and if they present any potential problems in fuel handling or engine operation.

3.2. Performance properties of unique FAME mixture

Measured properties for the unique FAME and a recently acquired soy biodiesel are detailed in Table 3. Soy biodiesel was selected for comparison because it is the dominant feedstock used in the United States [33]. Many of these properties are specified in ASTM D6751 Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels, and these requirements are also shown in Table 3. The unique FAME meets the numerical D6751 requirements after treatment with 50 ppm of TBHQ antioxidant, although it is not clear if it meets the definition of biodiesel in the standard (i.e., fuel comprising mono-alkyl esters of long-chain fatty acids derived from vegetable oils or animal fats) because of its production from glucose and the presence of β -hydroxy FAME. If FAME meeting the numerical property requirements of D6751 is to be produced commercially via glucose fermentation, we recommend that the biodiesel definition be updated to include this pathway. The presence of β -hydroxy esters is a more complex question. It would need to be shown that FAME containing substantial concentrations of these esters is within the scope of all test methods and that additional specification requirements are not needed before these could be allowed.

Cloud points are measured by cooling the fuel sample and detecting the temperature where crystals first appear. Cloud point is the most common metric used to determine a fuel's low-temperature operability limit. The appropriate cloud point for a fuel depends on the ambient temperature; therefore, no specific requirements are provided in D6751 other than that the cloud point must be reported. A cloud point that is not matched to the ambient conditions can lead to wax formation during overnight cooling and subsequent fuel filter plugging. The unique FAME and soy biodiesel have essentially the same cloud point (0.2 °C versus 0 °C), suggesting that the new FAME could be just as widely used as soy biodiesel in winter months.

Flash point is the temperature at which the gas phase above the fuel, in a closed container, is a flammable mixture of fuel and air. Flash point is a critical safety property for diesel fuels and must be above 52 °C for finished fuels. For B100 blendstock to be used in making biodiesel blends, flash point is set at a higher level of 93 °C minimum so that this

Table 1
Composition and properties of unique FAME and biodiesel samples.

Analysis	Units	Unique FAME (Sample 1)	Typical Soy Biodiesel ^a	Typical Tallow Biodiesel ^a	Typical Rapeseed Biodiesel ^a
FAME	% (w/w)	86	100	100	100
C12:1 FAME	% (w/w)	1.1			
C12:0 FAME	% (w/w)	1			
C13:0 FAME	% (w/w)	<0.1			
C14:1 FAME	% (w/w)	8.7			
C14:0 FAME	% (w/w)	19.5		2.6	
C15:0 FAME	% (w/w)	0.15			
C16:1 FAME	% (w/w)	39.8	0.2	2.6	0.1
C16:0 FAME	% (w/w)	15	11.6	24.3	4.2
C17:0 FAME	% (w/w)				
C17:0 cyclopropane FAME	% (w/w)	0.27			
C18:3 FAME	% (w/w)		5.9	0.9	8.4
C18:2 FAME	% (w/w)		53.8	4.4	21.5
C18:1 FAME	% (w/w)	0.8	23.7	42.4	59.5
C18:0 FAME	% (w/w)		3.9	18.2	1.6
β -hydroxy FAME	% (w/w)	9.2	–	–	–
C14:1 β OH FAME	% (w/w)	0.1			
C14:0 β OH FAME	% (w/w)	2.1			
C16:1 β OH FAME	% (w/w)	5.4			
C16:0 β OH FAME	% (w/w)	1.6			
Average fatty acid carbon #		14.55	17.60	17.07	16.53

^a Soy, tallow, and rapeseed compositions from [26].

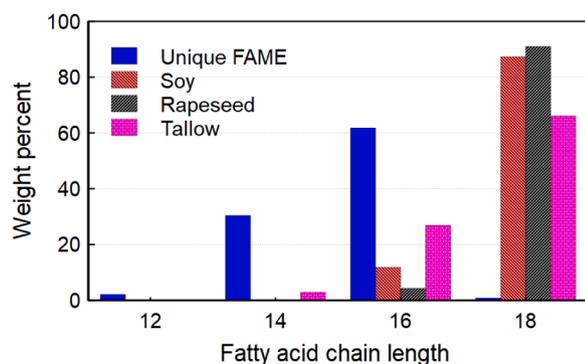


Fig. 2. Fatty acid chain length for unique FAME blendstock compared to conventional biodiesel from soy, rapeseed, and tallow.

Table 2
Results of impurity measurements for β -hydroxy FAME Sample 1.

Method	Sample code	β -hydroxy FAME blend
D664	Total acid number, mg KOH/g	Below detection
D874	Sulfated ash, mass%	0.013
D2709	Water and sediment, vol%	<0.01
Karl Fischer	Moisture, KF	123.4 \pm 4.1
D4530	Carbon residue, mass%	0.01
D6584	Free glycerin, wt%	<0.005
	Total glycerin, wt%	<0.05
	Monoglycerides, wt%	<0.1
	Diglycerides, wt%	<0.05
	Triglycerides, wt%	<0.05
D7111 Mod	Calcium, ppb	304
	Magnesium, ppb	<100
	Potassium, ppb	<1000
	Sodium, ppb	<1000
D4591	Phosphorus, ppm	<5

fuel is in the least hazardous category under National Fire Protection Association codes. A still higher flash point of 130 °C is required if flash point is used to ensure very low levels of residual methanol from the biodiesel production process. The unique FAME easily meets this higher requirement.

Engines are designed to operate on fuels within a particular range of distillation temperatures and viscosity. The maximum allowable values

Table 3
Property data for unique FAME and soy biodiesel compared to ASTM D6751 requirements. Reported experimental error is ASTM method repeatability (or 95 % confidence interval) or in house determined 95 % confidence interval for other methods.

	Method	Unique FAME	Soy biodiesel	D6751 requirement	Units
Cloud point	D5773	0.2 \pm 0.7	0 \pm 0.7	R eport	°C
Flash point	D6450	148 \pm 1.9	176 \pm 1.9	\geq 93	°C
T90	D1160	321 \pm 5	352 \pm 5	\leq 360	°C
Kinematic viscosity at 40 °C	D445	3.85 \pm 0.02	4.26 \pm 0.02 ^a	1.9–6.0	cSt (mm ² /s)
Density at 25 °C	D4052	0.8757 \pm 0.0002	0.8817 ^b	ns ^c	g/mL
Net heating value	D240	36.3	38	ns ^c	MJ/kg
Rancimat IP	EN15751	1.5 \pm 0.3 (4.2 \pm 0.4 ^d)	5	\geq 3	h
TAN	D664	<0.02	0.08 \pm 0.01	\leq 0.5	mg KOH/g
Peroxide content		23.8 \pm 4	–	ns ^c	ppm
Copper corrosion	D130	1A	1A	\leq 3	
ICN	D8183	56.7 \pm 1	52 \pm 1	\geq 47	–
YSI	Princeton	94.5 \pm 2.1	138.6 ^e	ns ^c	–

^a From [36].

^b From [34].

^c Not specified in standard.

^d With 50 ppm of TBHQ added.

^e Calculated—see text.

listed in Table 3 for compliance with D6751 have been shown to allow blends up to 20 vol% biodiesel that are fit for purpose. The unique FAME described here meets these requirements. However, there is concern that for higher level blends, or use of B100 as a fuel, the T90 and viscosity of current biodiesel is so high that the fuel will not fully evaporate, leading to in-cylinder carbon deposits and high lube oil dilution. Because of the shorter average chain length of the unique FAME sample, its T90 is more than 30 °C lower than that of the soy biodiesel, potentially providing some advantage in preparation of high-level blends or use of neat B100.

Some fuels can oxidize in storage or in the onboard fuel tank to produce gums and acids that are detrimental to engine performance and durability. Conventional FAME biodiesel can be moderately prone to oxidation because of the presence of polyunsaturated fatty acid chains that present a *bis*-allylic carbon atom. The relatively weak C—H bond at this carbon atom can break, leading to radical formation, and such radicals can react with the low levels of dissolved oxygen in the fuel—initiating the oxidation process [35]. An oxidation induction time measured using the EN15751 test (commonly referred to as Rancimat induction time) is used to control biodiesel oxidation stability, with a minimum 3-h requirement. The commercial soy biodiesel sample meets this requirement, likely because of the addition of antioxidant fuel additives. The unique FAME as received exhibits a low induction time of 1.5 h. This is unexpected because this sample contains only saturated and monounsaturated fatty acid chains. The addition of 50 ppm of the antioxidant TBHQ results in a passing induction time. Fig. 3a shows the response of the unique FAME for addition of TBHQ or BHT and comparison to results for soy biodiesel from the published literature [23,36]. The unique FAME is much more responsive than the soy biodiesel samples to both antioxidants. BHT is only marginally effective in soy biodiesel. While the species that are oxidizing in the unique FAME are unknown, this high response is likely because they are present at much lower concentration than the polyunsaturated FAME that makes up over 50 % of the soy biodiesel. Despite the poor stability of the as-received unique FAME, the low peroxide and TAN values, as well as the passing copper corrosion, indicate that this sample had not undergone an appreciable degree of oxidation.

Because of the unexpected low stability of the as-received unique FAME, an additional study was conducted using a modified version of the ASTM D4625 method. This method stores 400 mL of the fuel sample at 43 °C for many weeks in a container open to air. Results comparing the as-received unique FAME, TBHQ-treated unique FAME, and a soy biodiesel with <1 h initial induction time are shown in Fig. 3b for peroxide formation over 6 weeks of storage. The unique FAME sample oxidizes very slowly, and addition of TBHQ stops peroxide formation. A soy biodiesel with initial Rancimat of less than 1 h formed peroxide much more rapidly. Total acid number did not increase significantly over the course of this experiment. This result is consistent with a much lower concentration of oxidizing species in the unique FAME relative to soy biodiesel. Given that saturated and monounsaturated FAME are not easily oxidized, we speculate that the β -hydroxy components of the unique FAME sample are responsible for the observed oxidation on the Rancimat and D4625 tests. In any case, a detailed understanding of the cause of the observed oxidation will need to be developed before this

fuel can be successfully commercialized.

Mass and energy density are important properties related to fuel economy (km/L or mi/gal). The mass density of the unique FAME is almost the same as that of soy biodiesel. Energy density is slightly lower than for soy biodiesel because of the shorter average fatty acid chain length and the presence of β -hydroxy acids, leading to a higher O/C ratio and hence lower energy density.

CN is a measure of ignition delay—shorter ignition delay means higher CN. Diesel fuels must meet a minimum CN of 40 (ASTM D975) in the United States to ensure ease of starting, cold starting, and proper engine operation. CN is traditionally measured in an engine, but there are several alternative methods using constant-volume combustion chambers that are commonly used today. CN in this study was measured using one of these alternative methods, D8183, which reports an ICN. The ICN of the unique FAME sample is significantly higher than that of soy biodiesel, likely because the sample contains no di- or tri-unsaturates, which have low CN [26].

YSI is a measure of the intrinsic soot formation tendency of a sample. The measured YSI of the unique FAME sample is 94.5. Because a measured value does not appear to have been published for soy biodiesel, it was calculated as a mole fraction-based average using the analysis in Table 1 (using newly measured YSI values from this study, where available, and predicted values otherwise). YSI has been shown to blend linearly on this basis [38]. The value of 138.6 is significantly higher than that of the unique FAME sample, which seems reasonable given the higher average molecular weight, lower O/C ratio, and higher unsaturated content—all of which correlate with higher sooting tendency. A similar prediction for the unique FAME blendstocks yields a value of 105.8, in reasonable agreement with the measured value. Details of these calculations are in the SI.

3.3. Properties of blends with petroleum refinery diesel

Historically, biofuels have been blended into petroleum diesel at low to moderate levels. In the United States, research on biodiesel has focused on gaining market acceptance for blends up to 20 vol%. Because conventional soy biodiesel has higher kinematic viscosity, cloud point, CN, and T90 than conventional diesel, these properties are of interest. Biodiesel has also been shown to impart lubricity to blends [30]. Because biodiesel can be more prone to oxidation in storage, oxidation stability was also examined. Fig. 4 shows blending impact on kinematic viscosity of blends in Diesel A. Even at 50 vol% of the unique FAME blendstock, kinematic viscosity was well below the upper limit for conventional diesel fuel or for B20 blends of 4.1 mm²/s, and somewhat

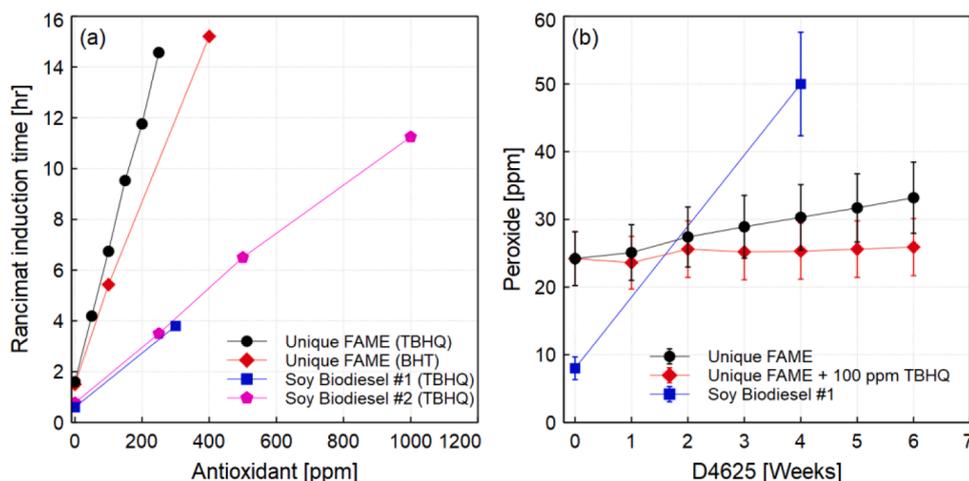


Fig. 3. (a) Rancimat induction time response to adding TBHQ or BHT into unique FAME and soy-derived biodiesel (Soy #1 [23] and Soy #2 [36]). Error bars are EN 15,751:2024 method reproducibility or 95 % confidence interval. (b) Comparison of D4625 oxidation stability results for unique FAME sample and soy biodiesel. Error bars are method 95 % confidence interval on peroxide measurement [37].

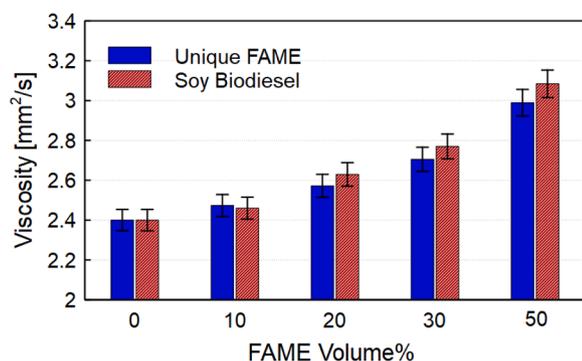


Fig. 4. Kinematic viscosity at 40 °C of blends of unique FAME or soy biodiesel with conventional diesel (Diesel A). Error bars are ASTM D445 method reproducibility or 95 % confidence interval.

lower than observed for a soy biodiesel blended into the same diesel fuel. Cloud point results for blending of the unique FAME blend compared to soy biodiesel are shown in Fig. 5. Unique FAME blends show lower cloud point than soy FAME blends, likely because of the shorter average chain length, even though the neat FAME samples have essentially the same cloud point.

Petroleum diesel is commonly treated with lubricity improver additives to meet the minimum lubricity requirement. Lubricity for blends of the unique FAME into a diesel fuel, treated to remove additives, is shown in Fig. 6. The unique FAME blend improved the diesel lubricity, as the wear scar diameter decreased from 520 μm for Diesel B, which is at the maximum allowable limit for diesel fuel, to 360 μm for the 10 % blend. Lubricity data for a soy biodiesel [39] show an even larger improvement. The smaller impact for the unique FAME may be caused by the very low levels of the monoglyceride and fatty acid impurities that have been shown responsible for most of the lubricity improvement observed for biodiesel [30].

Fig. 7 shows distillation results for blends of the unique FAME and soy biodiesel into Diesel A. The distillation T90 is controlled in the ASTM standards for diesel fuel and biodiesel blends to ensure full evaporation of the fuel. The maximum T90 for No. 2 diesel fuel is 338 °C (ASTM D975) and for biodiesel blends from 5 to 20 vol% is 343 °C. There are no standards for blends above 20 vol%. The T90 of Diesel A is 323 °C. Blending of soy biodiesel increases T90 but it remains within specification limits, even for blends above B20—in part because of the relatively low T90 of the base diesel fuel. The 80 % soy biodiesel blend boils at or above the base diesel T90 for a large fraction of the distillation curve. As expected, because of its much shorter chain length and lower T90 relative to soy biodiesel, the unique FAME has a much lower impact on the distillation curve, and no impact on T90. This may prove to be an advantage as biodiesel blend levels are increased to obtain lower carbon

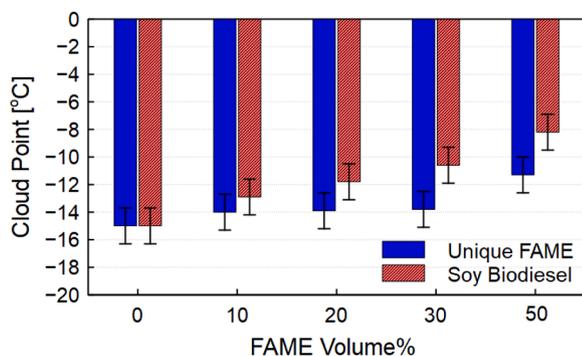


Fig. 5. Cloud point for blends of unique FAME and soy biodiesel with conventional diesel (Diesel A). Error bars are ASTM D5773 method repeatability or 95 % confidence interval.

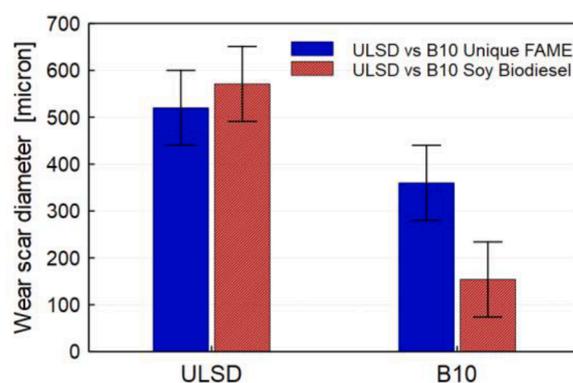


Fig. 6. Lubricity measured by high-frequency reciprocating rig for ultra-low-sulfur diesel (ULSD) (blue: clay-treated Diesel B; red: ULSD from [39]) and 10 vol% blends (B10) with the unique FAME blendstock into a clay-treated diesel fuel (Diesel B, blue) and soy biodiesel into ULSD data from reference [39] (red). Error bars are D6079 method reproducibility or 95 % confidence interval.

intensity in the finished fuel.

Cetane numbers (as ICN) for blends with conventional diesel are shown in Fig. 8. The soy biodiesel exhibits moderate synergistic blending—the blend CN is higher than predicted by a linear volumetric blending model by about 1 CN unit for blends in the 40–80 vol% range. The unique FAME exhibits an even larger synergistic effect—up to 4 CN units at 70 vol%. The same synergistic effects are observed when results are plotted on a molar concentration basis (see SI). The mechanism of this effect is the subject of ongoing investigation. Blend CN can be normalized as shown in the following equation:

$$\text{Normalized ICN} = \frac{\text{ICN}_{\text{mix}} - \text{ICN}_b}{\text{ICN}_a - \text{ICN}_b}$$

Where ICN_b and ICN_a are the ICN of the base fuel and additive, respectively. Normalized ICN accounts for the higher CN of the unique FAME blendstock, and Fig. 8 shows that both FAME blendstocks have a similar effect on a normalized basis. Nonlinear blending for CN is a poorly understood phenomenon that should be a focus area for future research.

Storage or oxidation stability was also measured for blends of the unique FAME at 30 vol% in diesel by measuring the Rancimat induction time as shown in Fig. 9. For the unique FAME with no antioxidant, blending with diesel resulted in a Rancimat IP that is essentially the same as that of the B100 (2.0 h vs 1.6 h). This is an unusual observation, as studies usually show that blending FAME with conventional diesel at levels in this range improves stability relative to the neat biodiesel [23, 36,40–42]. TBHQ was blended into the unique FAME B100 at 50, 200 or 1000 ppm and BHT was blended at 1000 ppm only. As shown in Fig. 3, these antioxidants were effective at increasing the IP for the unique FAME in neat form. For blends, adding 50 ppm of TBHQ to the B100 before blending increased 30 % blend Rancimat to 4.2 h. Higher TBHQ levels showed a larger effect. BHT was also highly effective at 1000 ppm.

3.4. Properties of β -hydroxy FAME

The unique FAME blend evaluated here has several properties that make it exceptional, including shorter average chain length and the presence of only unsaturated and monounsaturated fatty acid chains. However, the factor that is most distinguishing is the presence of FAME with a hydroxyl group at the β position. β -hydroxy esters have not been widely studied as fuels. We acquired two samples of relatively pure β -hydroxy esters. Sample 2 is 95 % purity methyl β -hydroxytetradecanoate (C14:0), and Sample 3 consists of a 98 % pure mixture of saturated and unsaturated (38 wt% C14:0 + 60 wt% C14:1) β -hydroxy

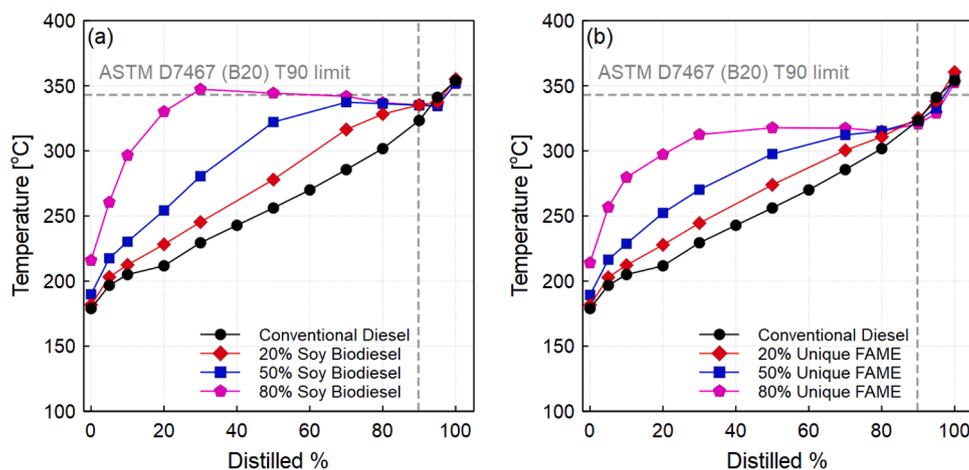


Fig. 7. Distillation results for (a) soy biodiesel and (b) unique FAME blends into Diesel A. Distillation of Diesel A was performed according to the D86 method, and distillation of blends by D2887 simulated distillation. Error bars (method repeatability) for T90 are less than ± 1.5 °C for these methods. Error of method D2887 for blends above B20 has not been determined.

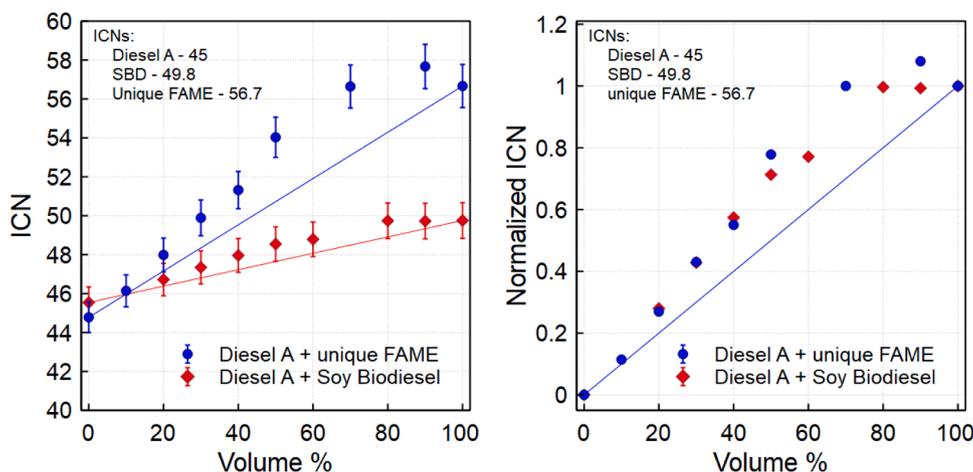


Fig. 8. ICN and normalized ICN for unique FAME and soy biodiesel (SBD) blended into conventional diesel (Diesel A). Error bars are method D8183 repeatability or 95 % confidence interval.

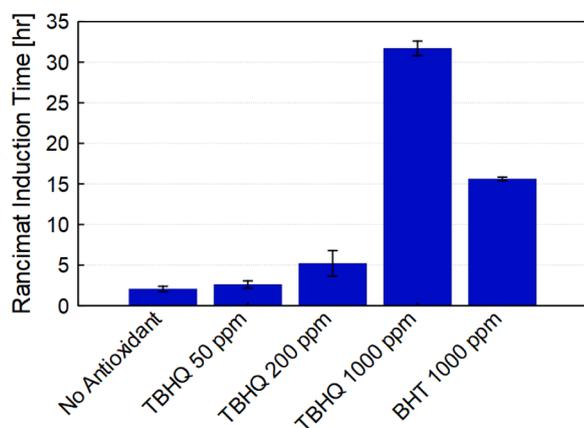


Fig. 9. Measured Rancimat induction times for 30 vol% blends of the unique FAME blendstocks in conventional diesel (Diesel A). Error bars indicate Rancimat (EN 15,751:2014) method repeatability or 95 % confidence interval. The antioxidants TBHQ or BHT were blended into the B100 at the levels shown in the chart prior to blending with conventional diesel.

Table 4

Property data for Samples 1 and 2, as well as methyl tetradecanoate for comparison (DSC and TGA experimental methodology and results reported in SI).

	Method	Sample 2 ^a	Sample 3 ^b	Methyl tetradecanoate	Units
Melting point	DSC	39	33	18.1 ^c	°C
Boiling point	TGA	326	–	295 ^d	°C
ICN	Predicted	45.6	–	68.4 ^e	–
YSI	Predicted	83.0	92.7 ^f	96.1 ^g	–
TAN as received	D664	1.51	2.81	–	mg KOH/g
Peroxide content as received		<5 ^h	3542	–	ppm

^a Methyl-3-hydroxy tetradecanoate, C14:0 β -hydroxy FAME;.

^b (38 wt% C14:0 + 60 wt% C14:1) β -hydroxy FAME;.

^c From [44];.

^d From [45];.

^e Measured in this study;.

^f Calculated from predicted values on molar basis;.

^g Measured by this study;.

^h Solid at room temperature. Dissolved 1:1 decane, measured peroxide content at 2.6 ppm (below detection).

methyl esters. The C14:0 and C14:1 esters are labeled as I and II, respectively, in Fig. 1. Certain measured and predicted properties for these samples are shown in Table 4, along with properties of methyl tetradecanoate for comparison.

The β -hydroxy methyl tetradecanoate (Sample 2) has higher melting temperature than methyl tetradecanoate, likely because of increased hydrogen bonding caused by the hydroxyl group. The mixed β -hydroxy C14:0 and C14:1 (Sample 3) does not as a mixture have a melting point but shows reduced melting transition temperature relative to Sample 2—in line with the lower melting points of unsaturated FAME. Boiling point of Sample 2 is higher than for methyl tetradecanoate—again because of the higher degree of hydrogen bonding of the hydroxyl group. Less obvious is the much lower CN of the saturated β -hydroxy C14:0 relative to methyl tetradecanoate. While this is based on a predicted value, the estimated error in the prediction is ± 6 CN units [43]—much smaller than the 22 CN unit difference in values for these compounds. The YSI value for the β -hydroxy C14:0 is somewhat lower than methyl tetradecanoate, likely because of the additional oxygen atom. The saturated/unsaturated β -hydroxy FAME mixture has very similar YSI to methyl tetradecanoate (93 versus 96), likely because of increased sooting tendency of the β -hydroxy C14:1 (predicted YSI 98.8). Measured and predicted YSI values are shown in the SI.

TAN and peroxide content of these samples are related to production method, storage and handling, and the inherent stability of the samples. The TAN of these samples is considered high (D6751 limits TAN for 100 % biodiesel to less than 0.5 mg KOH/g). Because of the low peroxide content of Sample 2, the high TAN does not appear to have been caused by oxidation during storage. For Sample 3, the peroxide content of over 3500 ppm is extremely high, and almost certainly implies that this sample has oxidized during storage and handling. In considering the oxidation stability of the unique FAME Sample 1—which would be expected to be quite stable given that it consists of only saturated and monounsaturated FAME—we stated the hypothesis that the β -hydroxy FAME is oxidizing in the liquid phase. The low peroxide content of Sample 2 compared to the high peroxide content of Sample 3 further suggests that β -hydroxy C14:1 (and β -hydroxy C16:1) are inherently unstable to oxidation. The high peroxide of Sample 3 supports but does not prove this hypothesis because we do not know the full details of how this sample was handled over time.

The melting point, boiling point, CN, and YSI were determined or predicted for different saturated β -hydroxy esters ranging from C6–C16, as shown in Table 5. Values for all properties increased with the chain length. Note that the predicted T_b for Sample 2 was 334 °C—in reasonable agreement with the value measured by TGA. For CN, the measured value for the C6 β -hydroxy ester was 8.1, in good agreement with the predicted value. DSC and TGA thermograms for the T_m and T_b measurements, respectively, are shown in the SI.

Fig. 10 shows the properties of different β -hydroxy esters compared

Table 5

Property data for different saturated β -hydroxy esters (fatty acid chain carbon number given in parentheses).

Sample	T_m , °C ^a	T_b , °C ^b	CN ^c	YSI
Methyl-3-hydroxy hexanoate (C6)	<−70	207	10.6	30.2
Methyl-3-hydroxy octanoate (C8)	−19.6	244	29.7	43.0
Methyl-3-hydroxy decanoate (C10)	−0.8	278	34.9	55.8
Methyl-3-hydroxy dodecanoate (C12)	19.4	308	40.3	69.8
Methyl-3-hydroxy tetradecanoate (C14) (Sample 2)	39	326	45.6	81.4
Methyl-3-hydroxy hexadecanoate (C16)	–	358	50.4	94.3

^a Melting points measured by DSC, results reported in SI.

^b Boiling points predicted using EPI Suite [46], except for Sample 2 measured by TGA.

^c CN and YSI predictions are from the cetane number [19] and YSI [22] prediction tools.

with their analogous conventional esters. Boiling and melting points of the β -hydroxy FAME are substantially higher than for conventional FAME of the same chain length, although the effect diminishes for T_b as chain length increases. These effects seem consistent with the higher degree of hydrogen bonding expected for the hydroxy esters. CN, on the other hand, is significantly lower for the β -hydroxy esters. In separate work, we investigated and compared the autoignition kinetics of conventional and β -hydroxy esters, where the hydroxyl group in the latter introduced alcohol-like combustion chemistry [47]. In the β -hydroxy esters, the main radical produced is on the carbon between the hydroxyl and ester groups. At low temperatures, this radical mainly reacted with O₂ to form HO₂ and an oxo-ester species (including both carbonyl and ester functional groups). This chain propagation pathway inhibited the β -hydroxy ester reactivity by competing with conventional low-temperature chain branching reactions, accounting for the lower CN [48]. YSI values are a comparison of entirely predicted values for the β -hydroxy esters and mostly measured values (except for C16) for the conventional esters. The comparison shows little difference in YSI for C6 to C10, but on average significantly lower YSI for the C12–C16 β -hydroxy esters that are more in the diesel boiling range. As there are no β -hydroxy esters in the YSI prediction model training data, a more definitive comparison will require additional data on these compounds.

4. Conclusions

The chemical composition and properties of a unique diesel-boiling-range FAME mixture containing β -hydroxy esters were evaluated. While this material cannot be considered biodiesel because of the presence of the β -hydroxy esters, it met all of the numerical requirements of ASTM D6751—the specification for B100 biodiesel. The use of an antioxidant additive was required for the fuel to have adequate oxidation stability—as was also the case for soy biodiesel. This was unexpected because the unique FAME contained no di- or tri-unsaturated fatty acid chains—which are known to be responsible for poor stability in conventional biodiesel. We speculate that monounsaturated β -hydroxy FAME is the source of this poor stability. Because of the significantly shorter fatty acid chains in the unique FAME relative to soy biodiesel, blends up to 80 vol% could be prepared while still meeting ASTM standard T90 limits for diesel fuel.

An evaluation of the properties of β -hydroxy esters showed that they have negative impacts on the unique FAME blend properties—increasing T90 and cloud point, while decreasing CN. The unsaturated β -hydroxy FAME may cause decreased oxidation stability. A biodiesel with the same average chain length as the unique FAME and the same level of saturated and monounsaturated chains—but without the β -hydroxy FAME—would likely have even better properties. While T90, cloud point, and oxidation stability are difficult to predict quantitatively from molecular structure, CN for a mixture of FAME can be calculated as a mass average, yielding a value of 61 for the hypothetical FAME mixture containing no β -hydroxy groups—5 CN units higher than the unique FAME.

The research reported here reveals some clear directions for future research on β -hydroxy ester containing FAME.

- The reaction mechanism leading to poor stability on the Rancimat induction time test must be revealed in detail. Additional studies using standard stability tests such as Rancimat and D4625 should be augmented with more fundamental measurements of oxidation or decomposition rates in both aerobic and anaerobic environments.
- Non-linear CN blending, as observed here, is also a poorly understood phenomenon. Antagonistic blending, where the blend CN is lower than would be predicted by a linear blending model, can at least be hypothetically explained by the lower reactivity component acting as a radical scavenger to slow down the autoignition of the higher reactivity component. However, to our knowledge, synergistic blending as shown in Fig. 8, has not been explained.

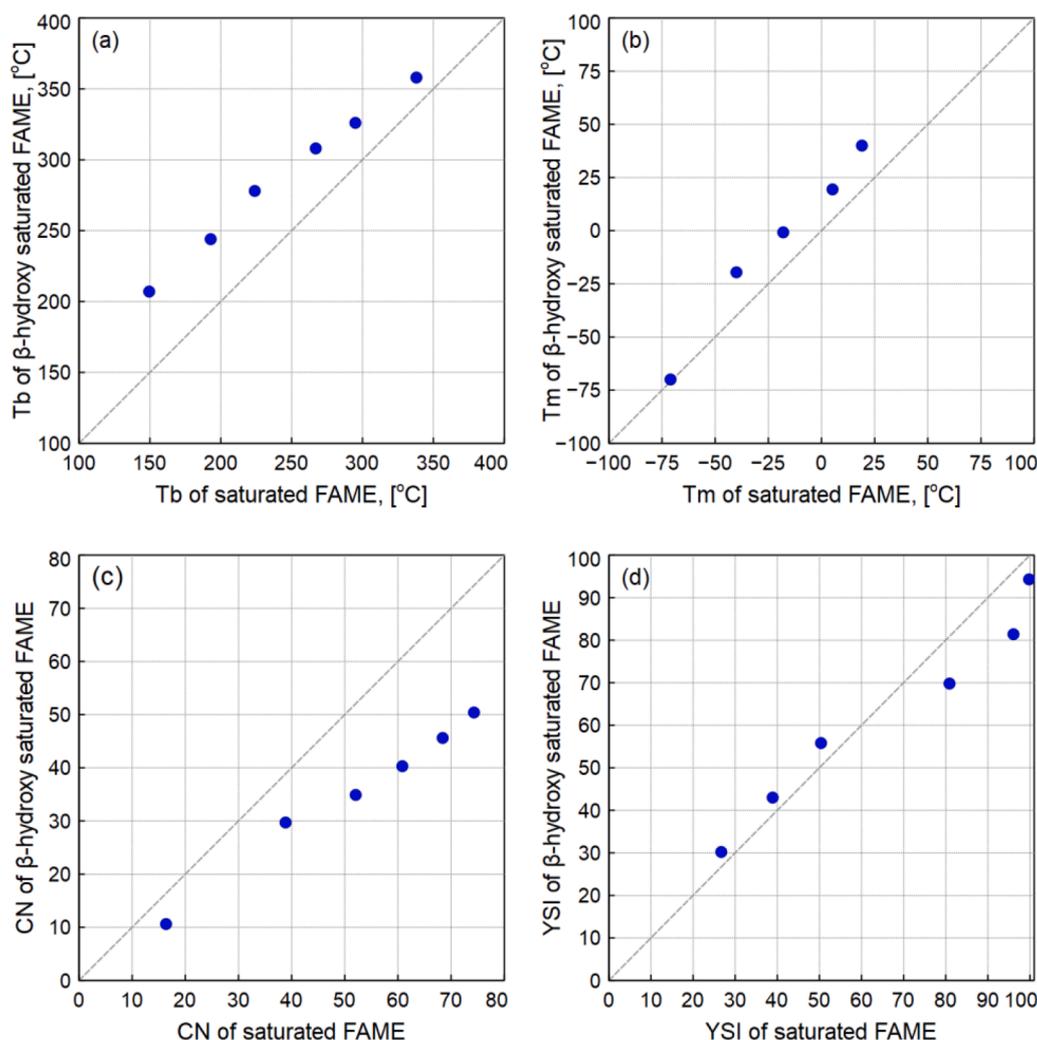


Fig. 10. (a) Boiling point (T_b), (b) melting point (T_m), (c) CN, and (d) YSI for different saturated β -hydroxy esters and their analogous conventional esters. Data for β -hydroxy esters from Table 5. Conventional ester data from [45] for boiling point, [44] for melting point, ICN values measured for this study (except for C16 from [49]), and YSI values measured or predicted for this study. See SI for conventional ester property values.

- Low-temperature operability is a key property for diesel fuels. Here we report cloud point for the unique FAME and blends into a diesel fuel. To fully understand the potential for any low-temperature issues, studies to examine blending into a range of both conventional and renewable diesels are needed, along with flow improver additive response studies.
- Engine combustion studies are likely to reveal attributes that cannot be predicted from fuel properties and lead to a much better understanding of many of the questions raised by this research.
- Analysis of production cost and scalability should be performed to understand commercial viability, as well as life-cycle analysis to understand sustainability.

Supporting information: The supporting information contains additional details on experimental methods, composition and properties of blendstocks (Sample 2, Sample 3, Diesel A, and Diesel B), YSI prediction for mixtures from pure component values, synergistic blending for CN on a molar basis, measurement of T_m and T_b for Samples 2 and 3, DSC results for T_m measurement of pure β -hydroxy esters, and properties of conventional esters (T_m , T_b , ICN, and YSI).

CRediT authorship contribution statement

Robert L. McCormick: Writing – review & editing, Writing –

original draft, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization. **Gina M. Fioroni:** Validation, Supervision, Methodology, Investigation, Data curation. **Samah Y. Mohamed:** Writing – original draft, Validation, Data curation. **Nimal Naser:** Methodology, Investigation. **Teresa L. Alleman:** Methodology, Investigation. **Seonah Kim:** Validation, Formal analysis, Conceptualization. **Ziyu Wang:** Methodology, Investigation. **Ying Lin:** Methodology, Investigation. **Yiguang Ju:** Methodology, Investigation, Conceptualization. **Kenneth Kar:** Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jfueco.2024.100120.

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