Oxidation Stability of Biodiesel Derived from Waste Catfish Oil

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ABSTRACT

The present work investigates the properties and oxidation stabilities of biodiesel derived from catfish oil (B100 CFO) generated by the fish processing plants in Vietnam. The composition and physicochemical properties of the B100 CFO were measured, including oxygen content (~ 11 wt%), viscosity ($4.5306 \text{ mm}^2 \text{ s}^{-1}$), density (0.8772 g cm^{-3}), peroxide value (28.13 mg kg^{-1}), heat of combustion (39.18 MJ kg^{-1}), and acid number ($0.12 \text{ mg KOH g}^{-1}$). Methyl oleate (46.44 wt%) was the dominant fatty acid methyl ester. B100 CFO was found to have poorer low-temperature quality than most vegetable oil derived biodiesels as determined by its fusion and crystallization properties. Novel modified ASTM D5304 and D2274 tests were employed to study the oxidation process of B100 CFO and the impact of oxidation time on its stability. Under ASTM 5304 test conditions, rapid O_2 consumption by B100 CFO occurred after 8 hours, behavior similar to commercial biodiesel stabilized with antioxidant additives. The influence of oxidation condition and time on the B100 CFO physicochemical properties and low-temperature qualities was also investigated according to ASTM methods. Three oxidation stages were identified in B100 CFO based on the peroxide value change during the modified ASTM D5304 and D2274 tests. B100 CFO was also found have superior oxidation stability in comparison with B100 derived from waste cooking oil. This is the first report on oxidation stability of B100 derived from catfish oil.

Keywords: biodiesel; fuel oxidative stability; distillate physicochemical properties; catfish oil.

Introduction

Environmental impacts of fossil fuel use have led to increased emphasis on producing alternative fuels from renewable feedstocks. Interest in biodiesel, a fuel comprised of mono-alkyl esters of long-chain fatty acids derived from vegetable oils or animal fats [1], continues to increase throughout the world motivated by its renewable and environmental benefits. In 2015, approximately 4.8 billion liters of biodiesel were produced in the U.S., and a similar amount, approximately 4.842 billion liters, was produced in the first ten months of 2016 [2]. As of October 2016, there were 93 biodiesel production facilities in the U.S. with total capacity exceeding 7.6 billion liters per year [2]. According to the U.S. Energy Information Administration 2015 report, approximately 4.257 million Mg of feedstock were used for biodiesel production. Over 70 % of these feedstocks, however, were virgin vegetable oil [2], which may impact food supply. In addition, one of the major obstacles to lowering biodiesel prices is the relative high cost of the oil feedstock. Biodiesel production costs can be reduced by using lower or negative value waste materials as feedstock with the added benefit of improving waste management. Low cost waste cooking oil [3], food processing waste [4], and by-product from wastewater treatment [5], have been recognized as potential feedstocks for biodiesel production.

Wastes generated from fish processing plants have attracted considerable attentions in recent years for biodiesel production. Approximately 50 wt% of harvested fish has been classified as waste during fish processing depending on the fish type and processing techniques [6]. For example, the eyes, tails, viscera, and flesh of fishes are discarded during the manufacturing process. Part of the fish waste can be ground and processed into fishmeal and pet food, which are low value products. For countries with large fish processing industries, it is possible to extract an oil byproduct from fish waste fraction for use as feedstock for biodiesel production. Recently, the catfish production and processing industries have been growing remarkably to accommodate the consumer demand. In 2013, farm-raised catfish was number eight on the “Top 10” list of fish and seafood consumed by Americans [7]. In 2014, the U.S. catfish industry processed approximately 136 Gg of catfish [7]. In Vietnam, striped catfish (*Pangasius*)

production reached 1.19 Tg in 2016, representing approximately 80 % of the total world production of Pangasius with an estimated export value of \$1.66 billion [8]. As most catfish processed in Vietnam is exported as fillets, the generation of waste fat is abundant, presenting a potential opportunity for biodiesel production.

Biodiesel is conventionally produced by transesterification. In this process, triglycerides (the main component of vegetable oils and animal fats) are converted to fatty acid methyl esters (FAME) in the presence of a catalyst by combining its acetyl part with methyl part of methanol. The glyceride group of triglyceride is replaced with hydroxyls to form glycerol as a by-product. Generally, two types of catalyst are used for biodiesel production depending on the composition of free fatty acid (FFA) in the feedstock. Base catalyst is used for bio-oil with <5 wt% FFA, whereas pretreatment with acid catalyst followed by the base catalyzed transesterification is used for bio-oils with >5 wt% FFA [9]. In the acid pretreatment process, the FFA is converted to monoesters in the presence of the acid catalyst [4]. Possible acid catalysts are organic sulphonic acid, sulphuric acid, phosphoric acid, and hydrochloric acid, and possible base candidates are sodium hydroxide, potassium hydroxide, carbonates, sodium amide, sodium hydride, potassium hydride and potassium amide [10]. Novel technologies have also been developed to improve biodiesel production process, e.g. ultrasound irradiation, microwave heating, co-solvent, and membrane reactor [11]. Among these new technologies, co-solvent technique has been regarded as promising approach to overcome miscibility problems in biodiesel synthesis. By using a co-solvent, the transesterification reaction is accelerated by allowing the mixture system to overcome some initial mass transfer resistance [11-13].

Although biodiesel has many advantages over conventional petroleum diesel, biodiesel is more prone to oxidation over time during storage or operation at high temperature due to the existence of unsaturated FAME. In general, the oxidation process of commercial biodiesel has three stages: (i) induction stage, in which free radicals react with antioxidant compounds preferentially rather than FAME; (ii) exponential stage, in which ~80-90 % antioxidants have been consumed and FAMEs start to rapidly react with

oxygen; (iii) termination stage, in which the rate of peroxide degradation exceeds the rate of peroxide formation and the fuel quality is significantly changed [14]. The oxidation stabilities of biodiesel derived from various bio-oil have been investigated before [15-25], and the major concern of oxidation stability is the tendency of fuels to react with oxygen, which highly depends on the composition of unsaturated FAMEs in biodiesel, especially compounds with bis-allylic methylene groups adjacent to the double bond [26, 27].

In this article, catfish oil from a fish processing plant was used to produce biodiesel by co-solvent transesterification [13]. The physicochemical properties and chemical composition of the catfish biodiesel were measured according to ASTM methods. Modified ASTM D5304 [28] and ASTM D2274 [29] were employed to study oxidation processes of the catfish biodiesel. The impacts of oxidation on fuel characteristics, such as composition, density, viscosity, peroxide value, acid number, heat of combustion, crystallization temperature were also investigated.

MATERIALS AND METHODS

Biodiesel Production

Catfish oil was obtained from Nam Viet Company, Tra Noc Industry Zone, Can Tho City, Vietnam. In general, the catfish were filleted and the bladder and stomach removed. The residue was ground and pressed to obtain an initial oil. This initial oil was subsequently heated at ~120°C under atmospheric pressure for ~25 min in a steam jacketed vessel to sterilize it and deactivate enzymes while avoiding thermal degradation. This heating process also promotes the coagulation of fish protein which can be separated from the oil. The heated oil was then centrifuged and filtered to produce the catfish oil (CFO) for biodiesel production (acid value: 2.56 mg KOH/g). A two-step process, (1) co-solvent transesterification of CFO and (2) purification of FAMEs [13], was used to produce the CFO biodiesel. The transesterification was performed at room temperature using a co-solvent comprised of methanol and acetone (2:1 w/w). Approximately 1 kg catfish oil was preheated in a flask at 60°C and stirred at 500 rpm

with a magnetic bar until the catfish oil formed a homogeneous liquid phase. Co-solvent solution, prepared by dissolving 10 g of KOH in 300 g co-solvent (200 g methanol and 100 g acetone), was added into the preheated catfish oil. The oil and solvent mixture was stirred at room temperature at 500 rpm for 4 h. The resulting mixture was transferred to a separatory funnel and stored overnight to allow the glycerol to separate from the biodiesel. After separation, excess methanol and acetone remaining in biodiesel was removed using a rotary evaporator at 60°C under vacuum for 1 h. The crude biodiesel was then washed three times to remove impurities using equal volumes of room temperature deionized water. After washing, water remaining in the FAME was removed by rotary evaporator at 70°C under vacuum for 30 min. The biodiesel was further dried with anhydrous sodium sulfate before testing and the anhydrous sodium sulfate was separated from biodiesel using nylon membrane filters (Whatman 0.8 µm pore size).

Chemical and Physical Properties

The composition of biodiesel was determined by gas chromatography/mass spectrometry (GC/MS). 1.5 µL fuel samples were dissolved in 1.5 mL hexane and analyzed using a Bruker 436-GC gas chromatograph and SCION-MS select, single quadrupole mass spectrometer (Bruker Corp., Billerica, MA). The GC was equipped with an Agilent DB1701 column (low/mid polarity, 60m, (14 %-cyanopropyl-phenyl)-methylpolysiloxane), with a 15 m guard-column before the back flush valve, and operated at a helium flow rate of 1.5 mL/min. FAME reference standards purchased from AccuStandard (AccuStandard, New Haven, CT) were used for identification of biodiesel components and composition determination. A LECO CHN628 (LECO Corp., St. Joseph, MI) was employed for the carbon, hydrogen, and nitrogen content determination of raw and stressed biodiesel samples. The furnace and afterburner temperature of the system was set at 950 and 850 °C, respectively. Approximately 60 mg of biodiesel was placed in a tin foil cup and then covered with ~300 mg Com-Aid (>99 % Al₂O₃, purchased from LECO Corp., Part No. 501-427) and sealed. The oxygen content of the biodiesel sample was calculated by subtracting the C, H, and N percentages from 100.

Differential scanning calorimetry (DSC) analyses were conducted using a TA Q2000 system (TA Instruments, New Castle, DE) equipped with an RCS90 temperature control which permits operation over the temperature range of -90 to 400 °C. A Tzero low-mass, hermetically sealed pan (TA Instruments, Part No. 901684.901 (lids) and 901670.901 (pans)) was selected for analyses with a sample mass of 2 to 3 mg. A purge gas of ultra-high purity nitrogen with a flow rate of 50.00 ml/min was regulated by a mass flow controller. Cooling and heating scans for biodiesel were conducted with the following program: (1) equilibrate at 30 °C, cool at 5 °C/min to -90 °C, and hold isothermally for 3 min; (2) heat at 5 °C/min to 30 °C, and hold isothermally for 3 min; (3) repeat step (1) and (2). The step (3), repeated cooling and heating scan, is conducted to ensure that the pan is well sealed and the curve is repeatable. The cooling scan was analyzed to determine the crystallization onset temperature (FO) and the crystallization peak temperature (FP), which reflect the low-temperature quality of biodiesel.

An Anton Paar SVM3000 Stabinger Viscometer (Anton Paar USA Inc., Ashland, VA) was used to measure the viscosity and density of neat and stressed biodiesel samples at temperatures according to the ASTM D7042 method [30]. The accuracy of the viscometer was tested with a certified viscosity standard oil (APN26, Paragon Scientific Limited, UK) and the measurement repeatability was ± 0.1 % of reading for viscosity, ± 0.0002 g/cm³ for density, and ± 0.005 °C for temperature. A Parr 6200 Isoperibol Calorimeter (Parr Instrument Company, Moline, IL) was used to measure the heat of combustion, i.e. heating value, based on the ASTM D4809 method [31]. The heat of combustion reported in this study is the high heating value (HHV). ASTM D3703 [32] and ASTM D974 [33] were employed to determine the peroxide value (PV) and acid number (AN) of biodiesel, respectively. PV is an indicator of the quantity of oxidizing constituents present in the fuel samples that influence various parameters, such as density and viscosity.

Oxidative Stabilities

Modified D5304 [28] and D2274 [29] methods were employed to stress the biodiesel and investigate its oxidative stability. The modified ASTM D5304 method stresses the biodiesel in a pure oxygen atmosphere at 90 °C and 800 kPa and investigates the oxygen consumption by continuously monitoring the pressure change of the pressure vessel for up to 40 h. ASTM D2274 is an accelerated method developed to quantify the oxidative stability of distillate fuel oil [34-36]. This method requires an elevated temperature, 95 °C, and continuous exposure to a 3 L/h, bubbled-flow of pure oxygen at ambient pressure. In addition, the variation of fuel physicochemical properties was determined as a function of time after the sample was subjected to modified ASTM D5304 and D2274 test conditions. For ASTM D2274 testing, ~40 ml fuel samples were removed from the oxidation cell at each time interval, cooled to room temperature in the dark, and subjected to property analysis. The details of these two methods are described in previous paper [37, 38].

RESULTS AND DISCUSSION

Physicochemical Properties

The physicochemical properties of B100 derived from catfish oil (CFO) were measured and compared with B100 derived from waste cooking oil produced by Big Island Biodiesel (Kea`au, HI) and petroleum ultra-low sulfur diesel (ULSD) provided by the United States Navy Supply Center (Patuxent River, MD). Figure 1 shows the GC/MS scan of B100 CFO, comprising seven peaks ranging in the retention time from 27 to 49 mins. The seven major compounds were identified as methyl myristate (MeC14:0), methyl palmitoleate (MeC16:1), methyl palmitate (MeC16:0), methyl oleate (MeC18:1), methyl linoleate (MeC18:2), methyl stearate (MeC18:0), and methyl linolenate (MeC18:3) in the following concentrations: 3.43 wt% MeC14:0, 1.67 wt% MeC16:1, 27.89 wt% MeC16:0, 46.44 wt% MeC18:1, 10.85 wt% MeC18:2, 8.38 wt% MeC18:0, and 1.33 wt% MeC18:3. These data indicate that the B100 CFO contains approximately 60% unsaturated FAMES and has a different composition from the commercial B100

derived from waste cooking oil (B100 WCO) investigated previously [3]. The B100 WCO contained five major compounds, i.e. 21.22 wt% MeC16:0, 43.56 wt% MeC18:1, 19.98 wt% MeC18:2, 11.57 wt% MeC18:0, and 3.64 wt% MeC18:3 [3].

Two indicators of fuel stability are provided by this analysis. Based on the lower concentration of unsaturated esters, B100 CFO (~60 wt%) is expected to be more stable than the B100 WCO (~67 wt%). A second indicator of stability of biodiesels is the concentration of unsaturated esters with bis-allylic methylene groups adjacent to the double bond. These compounds are very unstable under elevated temperature and/or pressure conditions in the presence of oxygen [23, 39]. The concentration of FAMES with this double bond structure in B100 WCO, ~ 24 wt%, doubles that in B100 CFO, ~12 wt%.

Table 1 compares the physicochemical properties of the B100 CFO with B100 WCO. Both meet the ASTM D6751 specification [1], — kinematic viscosity (ν) in the range of 1.9 - 6 mm² s⁻¹ at 40 °C and acid number (AN) < 0.5 mg of KOH/g. The high heating values (HHV) of B100 CFO and B100 WCO are also very similar, but both are lower than that of ULSD, 44.96 ± 0.18 MJ kg⁻¹. The density and kinematic viscosity of B100 CFO and WCO are both higher than that of ULSD, as shown in Figure 2. As biodiesel is commonly used for ground transportation in pure form (B100) and as 5, 7, or 20% blends with petroleum diesel, blending B100 CFO or WCO with ULSD increases the overall kinematic viscosity or density of the diesel blends.

The low temperature properties of B100 CFO were also investigated using DSC. Generally, biodiesel is susceptible to performance problems owing to the cold weather phase change of FAMES with high melting temperature. Figure 3 displays the heating and cooling scans of B100 CFO and B100 WCO. Three phase transitions were detected in B100 CFO and WCO samples, which are exhibited by the three distinct peaks during both the cooling and heating scans. The fusion processes of all of the FAMES present in the B100 CFO and WCO have been investigated before, and the melting temperatures of these

FAMES are (1) MeC14:0 19 °C, (2) MeC16:1 -34.10 °C, (3) MeC16:0 30 °C, (4) MeC18:1 -19.9 °C, (5) MeC18:2 -35 °C, (6) MeC18:0 39.1 °C, and (7) MeC18:3 -45.5 °C [40, 41]. The crystallization processes of MeC16:0, MeC18:0, and MeC18:1 were previously studied and their crystallization onset temperatures (FO) are 24.4, 33.7 and -40.6 °C, respectively [42]. As shown in the B100 WCO cooling scan, the first crystallization peak (FP at ~7 °C) spans a wider temperature range, approximately 40 °C, compared to the ~10 °C width observed for the -44 and -72 °C peaks. The phase transition of peak 1 is primarily induced by the crystallization of unsaturated FAMES with a single double bond and saturated FAMES. These include MeC14:0, MeC16:1, MeC16:0, MeC18:1, and MeC18:0. The subpeak at ~1°C is induced by the MeC14:0 and MeC16:1 present in the B100 CFO. Peaks 2 and 3 correspond to the phase change of FAME mixtures with polyunsaturation, i.e. MeC18:2, and MeC18:3. Table 2 compares the crystallization FO and FP of B100 CFO and B100 WCO on the cooling scan. Although FO and FP of peak 3 in the B100 CFO curve are lower than that of B100 WCO, B100 WCO exhibits better cold weather qualities owing to the lower FO and FP values of the first and second peaks. The phenomenon is mainly contributed by the higher composition of unsaturated FAMES in B100 WCO, indicating that biodiesel derived from animal fats may have inferior low-temperature qualities compared to biodiesel derived from vegetable oil. B100 derived from soybean, rapeseed, and sunflower seed [43] have better low temperature qualities than B100 WCO. B100 derived from peanut oil is an exception, as it is known to have poorer low temperature qualities than that of B100 CFO [43].

The preliminary property analysis results indicate that raw B100 CFO has superior stability in comparison with B100 WCO, whereas the low temperature quality of B100 CFO is inferior to that of B100 WCO. This indicates that higher concentration of saturated FAMES coupled with lower concentration of unsaturated FAMES, especially those with bis-allylic methylene groups adjacent to the double bond, has adverse effect on biodiesel stability and low temperature performance. A higher concentration of saturated FAMES would result in higher stability of biodiesel but lower low-temperature quality. Although AN and kinematic viscosity of B100 CFO meet the ASTM D6751 specification and are similar

to that of B100 WCO, the HHV of B100 CFO is lower than that of petroleum diesel ULSD, indicating that blending B100 with ULSD would result in a fuel blend with lower HHV.

ASTM D5304 Testing

ASTM D5304 was employed to investigate oxidation of B100 CFO. Although this method was initially developed to measure the insoluble formation in fuel samples under stressed conditions, (i.e. 90 °C and pure oxygen at 800 kPa), the modified method aims to investigate the oxidation process by characterizing the oxygen consumption of the fuel samples. This entailed monitoring the pressure in the oxidation vessel and calculating the percentage of oxygen remaining. Figure 4 displays the oxygen consumption of B100 CFO after 8, 16 and 40 h tests, compared with that of commercial B100 WCO with and without antioxidant. The antioxidant added in the commercial B100 WCO is approximately 0.04 % v/v Eastman BioExtend™ 30 solution. Manufacturer product literature indicates that the temperature of the biodiesel should not exceed 100 °C after the BioExtend™ 30 has been added [44]. No antioxidants were added in B100 CFO. As with B100 WCO with antioxidant, the oxygen consumption rate of B100 CFO is relatively slow until the breakpoint is reached, approximately 8 h, and the oxygen consumption rate is significantly accelerated after the breakpoint. However, the breakpoint of B100 WCO without antioxidant is reached merely after ~1 h test, which demonstrates that the B100 CFO has superior oxidation stability compared to B100 WCO. Stavinoha and Howell [45] reported similar phenomena with the oxygen consumption rate of B100 samples accelerating after ~1 h. The oxidation process of biodiesels before reaching the breakpoint is dominated by the reaction of free radicals with antioxidant. Vitamin E is a group of lipid soluble compounds that protect polyunsaturated lipids present in organisms against oxidation [46, 47] and has been shown to be thermally stable at temperatures of 180 °C [48]. Although commercial antioxidant was not added to the B100 CFO, natural antioxidants present in fresh catfish, e.g. vitamin E [49], protect polyunsaturated FAME against oxidation. Unlike the B100 CFO, antioxidants existing in cooking oil were consumed during the cooking process with little present in the B100 WCO. This results in the very short O₂ consumption induction period, ~1 h, of B100 WCO without antioxidant.

Changes in fuel composition and physicochemical properties were also investigated after 8, 16, and 40 h ASTM D5304 tests. Figure 5 shows the composition change of B100 CFO after ASTM D5304 tests. Concentration change was observed for MeC14:0, MeC16:0, MeC18:1, and MeC18:2. The concentrations of MeC14:0 and MeC16:0 slightly increase with oxidation time, which is caused by the oxidation and decreasing concentrations of MeC18:1 and MeC18:2. In addition, the oxidation and degradation rates of MeC18:2 are faster than that of MeC18:1, as reflected by the steeper slope of the MeC18:2.

The composition change of B100 CFO after stressing the fuel was accompanied by observed differences in properties. Table 1 compared the properties of B100 CFO before and after ASTM D5304 tests. The fuel density and kinematic viscosity show little change after 8 h of ASTM D5304 test conditions owing to coexistence of biodiesel degradation and polymerization reactions. The degradation reactions form small molecular weight substances, such as acids, aldehydes, alcohols, ketones, and peroxides [26], whereas the polymerization reactions form conjugated large molecules and/or polymers by the Diels-Alder reaction between the conjugated diene groups in the chain and polyunsaturated olefinic groups from nearby chains [23]. The coexistence of these two reactions was also reflected in the DSC results discussed below. After the breakpoint is reached, the oxidation reaction is dominated by the polymerization reaction, which is reflected by the significant increase of density and viscosity after stressing the B100 CFO for 16 and 40 h. The B100 CFO oxidation does not have significant impacts on HHV, with the difference after 40 h ASTM D5304 of <2 %.

The impacts of oxidation on peroxide value (PV) and AN are significant. The AN increased over an order of magnitude after 40 h test, and the PV increased over 35-fold after 16 h test. It is worth noting that the PV after 40 h test is lower than that of 16 h test, which indicates that the B100 CFO is already in the termination stage of the oxidation process after 40 h test. The change in fuel property is also evidenced by the change of oxygen content determined by the elemental analysis. The oxygen content of B100 CFO increased from ~11 % to ~13 %.

The impacts of oxidation on low temperature qualities of biodiesel were also investigated. Figure 6 shows the DSC cooling scan of B100 CFO before and after ASTM D5304 tests. The stressed B100 CFO samples have better low temperature characteristics compared to the parent B100 CFO. The FP of the first phase change in the cooling scans of B100 CFO after 8, 16, and 40 h tests are 7.13, 6.49, and 6.68 °C, respectively, which are all lower than that of parent B100 CFO, 7.97 °C. Similar phenomena were apparent at the second phase change in stressed B100 CFO with FP values of -45.64, -48.99, and -52.18 °C observed for the 8, 16, and 40 h test, respectively. All are lower than that of the unstressed parent sample, -43.85 °C. The lower FO and FP values are induced by the formation of small molecular weight substances with lower crystallization temperature [26] in the induction stage. After induction, the oxidation reaction is dominated by the Diels-Alder reaction, negatively affecting the low temperature behavior, as illustrated by the increased FP of the first phase change in the cooling scans of B100 CFO after the 40 h test (6.68 °C) compared to that after the 16 h test (6.49 °C).

The results obtained from ASTM D5304 testing are consistent with preliminary property analysis results. B100 CFO is more stable than B100 WCO owing to the higher concentration of saturated FAMES in B100 CFO. The stability of raw B100 CFO is even similar as that of B100 WCO with commercial antioxidant, as natural antioxidants, e.g. Vitamin E, usually exist in catfish oil feedstocks. The coexistence of degradation and polymerization reactions was also reflected by property changes of B100 CFO after ASTM D5304 testing. The PV result also illustrates that the B100 CFO is in the termination stage of the oxidation process after 40 h.

ASTM D2274 Test

Extended duration ASTM D2274 tests were conducted to investigate the impacts of oxidation time on biodiesel properties. B100 CFO was oxidized for periods up to 96 h at 95 °C under continuous flow of pure oxygen. This modified method focused on studying the fuel properties change after oxidation instead of measuring the insoluble formation. Figure 7 shows the composition change of the degrading B100 CFO over the extended ASTM D2274 tests. As expected, the concentration of all unsaturated FAMES, i.e.

MeC16:1, MeC18:1, MeC18:2, and MeC18:3, decreased with time owing to the oxidation of these compounds. MeC18:3 and MeC18:2 disappeared in the fuel sample after 16 and 72 h oxidation, respectively. This also resulted in increased concentrations of saturated esters, i.e. MeC14:0, MeC16:0, and MeC18:0. As illustrated by the slope of linear regression, the oxidation reaction rate of MeC18:2 is faster than that of MeC18:1 due to the existence of bis-allylic methylene groups adjacent to the double bond in MeC18:2 structure. Similar trends were also observed in ASTM D5304 tests of B100 CFO and ASTM D2274 tests of B100 WCO [3]. As MeC18:1, MeC16:1 also reacted with oxygen, its concentration dropped to 0 % after 96 h test. Figure 8 displays the change in the B100 CFO oxygen content during the extended ASTM D2274 tests. The oxygen content was calculated based on measurements of carbon, hydrogen, and nitrogen content of the sample. As with the results obtained from the ASTM D5304 tests, the oxygen content of stressed B100 CFO increased with test duration, increasing over 7 % (absolute) after 96 h.

The extended duration ASTM D2274 testing had critical impacts on physicochemical properties of B100 CFO. Figure 9 and 10 displays the kinematic viscosity, density, HHV and AN change during the test. As with B100 WCO [3], the kinematic viscosity, density and AN of B100 CFO increased with oxidation time, whereas the HHV decreased with oxidation time. After 96 h, the density and kinematic viscosity increased approximately 8 % and 300 % (relative), respectively, whereas the AN values were an order of magnitude higher. The HHV reduction of B100 CFO, approximately 12 %, is caused by the increased oxygen content in polymerized product, as demonstrated by the elemental analysis shown in Figure 8. The PV, shown in Figure 11, varies with oxidation time. The PV of B100 CFO reached a maximum, 3,639.8 mg kg⁻¹, after approximately 32 h of extended ASTM D2274 testing indicating that thereafter, the oxidation reaction transitions from the exponential stage to the termination stage. This is in good agreement with the oxidation mechanism of polyunsaturated fatty acid esters proposed by Christensen and McCormick [14]. A similar peak was also observed by the extended ASTM D2274 tests of B100 WCO at ~30h with the maximum value of 4,141.8 mg kg⁻¹ [3], which is approximately ~14 % higher than

that of B100 CFO. The higher PV of B100 WCO is mainly induced by the higher composition of unsaturated FAME in B100 WCO, especially FAMES with polyunsaturated double bonds.

The impacts of extended ASTM D2274 testing on low temperature qualities of B100 CFO were also investigated. Figure 12 shows the crystallization process of B100 CFO after extended duration ASTM D2274 tests. Similar as was observed in the ASTM D5304 testing, the FP of lower temperature peak, reflecting the existence of polyunsaturated FAME, decreases with oxidation time, and disappears after approximately 72 h oxidation. This is consistent with the results obtained in the GC/MS analysis, in which the concentrations of MeC18:2 and MeC18:3 were below the detection limit after 72 and 16 h oxidation, respectively. Figure 11 shows the relationship of the higher temperature FP and oxidation time. The low temperature quality of B100 CFO for the first 40 h of oxidation is similar as that of the parent sample and this could be due to a balance between degradation and polymerization reactions. The low-temperature quality deteriorates thereafter and may be attributed to the increased formation of polymers in exponential and determination stages. It is also worth noting that the decrease in polyunsaturated FAMES concentration appears correlated with the decrease of crystallization enthalpy of peak 2.

Results obtained by stressing B100 CFO using the ASTM D2274 method illustrate that all unsaturated FAMES reacted with oxygen. Oxidation reaction rates of FAMES with bis-allylic methylene groups adjacent to the double bond structure were faster than those of FAMES with a single double bond. The overall oxygen content of B100 CFO also increased owing to the continuous oxidation of unsaturated FAMES. Significant change was observed in other properties, including viscosity, density, HHV, AN, and PV. The change of PV demonstrated the existence of three stages in the biodiesel oxidation process. The low temperature quality of B100 CFO was also affected by degradation and polymerization reactions of unsaturated FAMES.

CONCLUSION

Waste catfish oil generated by fish processing plants in Vietnam was used to produce biodiesel by a co-solvent transesterification method. The properties of the purified B100 derived from catfish oil (CFO) was measured and compared to that of commercial B100 derived from waste cooking oil (WCO). The results indicate that the composition of B100 CFO is different from that of B100 WCO, which contributes to differences in their physicochemical properties and low-temperature qualities. Generally, the B100 CFO is more stable than the B100 WCO, as the unsaturated FAMES of B100 CFO account for approximately 60 wt%, which is ~7 % (absolute) lower than that in B100 WCO. The better stability of B100 CFO is also due to the lower composition of FAMES with polyunsaturated double bonds. However, B100 CFO has inferior low-temperature qualities compared to that of B100 WCO owing to the higher composition of saturated FAMES. The influence of oxidation on B100 CFO properties were also investigated by conducting modified ASTM D5304 and D2274 tests. Both ASTM D5304 and D2274 tests produced significant changes in physicochemical properties (viscosity, density, AN, PV, HHV) and reduced low-temperature qualities. The oxidation reaction in both tests also significantly increased the oxygen content of B100 CFO. The ASTM 2274 oxidation of B100 CFO reached the termination of the peroxide propagation stage after ~32 h as reflected by the changing shape of PV curve. Although no commercial antioxidant was added to B100 CFO, it had nearly identical oxidation stability as B100 WCO that contained an antioxidant additive. Waste catfish oil from fish processing plants holds potential for commercial biodiesel production in Vietnam.

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483 **Figure Captions**

484 **FIGURE 1.** GC/MS chromatogram of B100 CFO.

485 **FIGURE 2.** Kinematic viscosity and density of B100 CFO, B100 WCO, and ULSD.

486 **FIGURE 3.** DSC heating and cooling curve of B100 CFO and B100 WCO.

487 **FIGURE 4.** Oxygen consumption of B100 samples under ASTM D5304 test conditions.

488 **FIGURE 5.** Composition change of B100 CFO samples under ASTM D5304 test conditions.

489 **FIGURE 6.** DSC cooling curve after stressing B100 CFO for different test durations using ASTM D5304
490 techniques.

491 **FIGURE 7.** Composition change of B100 CFO after extended duration ASTM D2274 tests lasting up to
492 96 hours.

493 **FIGURE 8.** Oxygen content change of B100 CFO after extended duration ASTM D2274 tests.

494 **FIGURE 9.** Kinematic viscosity (measured at 40°C) and density (measured at 15°C) of B100 CFO after
495 extended duration ASTM D2274 tests.

496 **FIGURE 10.** Acid number and higher heating value change of B100 CFO after extended duration ASTM
497 D2274 tests.

498 **FIGURE 11.** Peroxide value and peak 1 crystallization temperature (FP) of B100 CFO after extended
499 duration ASTM D2274 tests.

500 **FIGURE 12.** DSC cooling curve after extended stressing of B100 CFO using ASTM D2274 methods.

501

Table 1. Physicochemical properties of B100 derived from CFO before and after modified D5304 tests.

Properties ^a	B100 WCO ^b	B100 CFO	D5304 8 h	D5304 16 h	D5304 40 h
ρ at 15°C (g cm ⁻³)	0.8778 ± 0.0001	0.8772 ± 0.0001	0.8778 ± 0.0000	0.8808 ± 0.0000	0.8845 ± 0.0000
ν at 40°C (mm ² s ⁻¹)	4.3725 ± 0.0016	4.5306 ± 0.0016	4.5608 ± 0.0008	4.8068 ± 0.0016	5.0994 ± 0.0031
PV (mg kg ⁻¹)	16.59 ± 0.49	28.13 ± 0.64	281.20 ± 10.77	985.75 ± 14.24	707.87 ± 5.84
HHV (MJ kg ⁻¹)	39.17 ± 0.04	39.18 ± 0.05	39.31 ± 0.19	39.02 ± 0.10	38.72 ± 0.11
AN (mg of KOH/g)	0.23 ± 0.01	0.12 ± 0.00	0.25 ± 0.00	0.85 ± 0.05	3.38 ± 0.01
O ₂ % w/w	11.7789 ± 0.8468	10.9811 ± 1.0628	11.9819 ± 0.8863	11.4952 ± 0.9203	12.7993 ± 0.4653

^a all values are the mean of three measurements and the uncertainty is the standard deviation.

^b reference [3]

Table 2. Summary DSC phase change of B100 derived from CFO and WCO.

Peak Number	FO (CFO)/°C ^{a,b}	FP (CFO)/°C ^{b,c}	FO (WCO)/°C ^{a,b}	FP (WCO)/°C
Peak 1	7.97	7.26 and 0.87	5.56	3.97 and 5.28
Peak 2	-43.85	-45.22	-49.72	-52.01
Peak 3	-72.45	-74.25	-63.06	-66.15

^a FO = crystallization onset temperature

^b The uncertainty of the temperature measurement is ± 0.10 °C

^c FP = crystallization peak temperature

Figure 1

Figure 2

Figure 3

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