

BIODIESEL FROM WASTE SALMON OIL

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ABSTRACT. *Salmon oils separated from salmon processing waste and hydrolysate and their derived methyl esters were analyzed and compared with corn oil and its methyl ester. These materials were characterized for their fatty acid profiles, viscosity, volatility, thermal properties, low temperature properties, oxidative stability, and heating value. The salmon oil methyl esters contained 26.64% saturated fatty acid methyl esters compared to 13.68% for corn oil methyl ester. Salmon oil methyl esters also contained relatively high concentrations of eicosapentaenoic acid (C20:5) and docosahexaenoic (C22:6) acid methyl esters. Despite these differences in fatty acid composition, salmon and corn oil methyl esters had comparable physical properties. In addition, the methyl esters produced from salmon oils extracted from fish processing by-products and hydrolysate showed little difference in their physical properties.*

Keywords. *Biodiesel, Fish by-product, Salmon oil.*

Plant and vegetable oils have been used as alternative fuels for a number of years. They are readily available and renewable materials. However, oils have a number of disadvantages when used directly as a fuel source (Ma and Hanna, 1999; Srivastava and Prasad, 2000; Demirbas 2003; Meher et al., 2006). First, they have high viscosities that are at least ten times higher than diesel fuel. High viscosity leads to poor fuel atomization during the injection process. Moreover, oils can polymerize and have low volatility, resulting in deposit formation in engines as well as incomplete combustion.

One way to overcome problems of oils as diesel fuels is by conversion of oils into biodiesel (i.e., fatty acid alkyl esters). Various plant and vegetable oils have been converted to biodiesel, including soybean, rapeseed, canola, sunflower, and palm oils (Ma and Hanna, 1999; Srivastava and Prasad, 2000; Fukuda et al., 2001; Meher et al., 2006). In the U.S., most research into biodiesel has focused on soybean oil, whereas

most research in Europe has focused on rapeseed oil. The reason is that these are the predominant oil seed plants grown in those areas. Less expensive feedstocks, such as spent frying oil and grease, have also been considered as alternative sources for biodiesel production (Muniyappa et al., 1996; Canakci and Van Gerpen, 2001; Lee et al., 2002; Zhang et al., 2003; Dmytryshyn et al., 2004; Tashtoush et al., 2004; Encinar et al., 2005).

Most research on biodiesel has focused on using plant-based oils as feedstocks. There has been much less research on converting animal-based oils into biodiesel (Muniyappa et al. 1996; Canakci and Van Gerpen, 2001; Lee et al., 2002; Kato et al., 2004; Tashtoush et al., 2004). One potential source of oil is fish oil from the Alaskan fishing industry. It has been estimated that over a million tons of fish by-products are generated annually from the fishing industry in Alaska (Crapo and Bechtel, 2003). Some of these by-products are converted into fish meal and oil, but approximately 60% are not utilized (Crapo and Bechtel, 2003). The major fish by-products include fish heads, viscera, and some frames, with much of the oil stored in the head.

Fish by-products can also be converted into hydrolysate through hydrolysis. Hydrolysis involves multiple enzyme and heat treatments to break down proteins into smaller peptides. The final hydrolysate product is usually stabilized by acidification and can be used as fertilizer or as feed ingredients. Hydrolysates of fish by-products contain a significant amount of oil, which can be extracted and converted into biodiesel.

The main objective of this study was to evaluate thermal, rheological, thermal stability, combustion, and cold flow properties of two types of salmon oil and their derived methyl esters. One oil sample, termed non-acidified oil, was extracted from fresh salmon by-product, and the other oil sample, termed acidified oil, was extracted from the hydrolysate made from the salmon by-product. Corn oil and its derived methyl ester were also evaluated as a comparison.

Submitted for review in December 2006 as manuscript number PM 6781; approved for publication by the Power & Machinery Division of ASABE in April 2008. Presented at the 2006 ASABE Annual Meeting as Paper No. 066157.

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MATERIAL AND METHODS

METHYL ESTER PRODUCTION

Methyl esters were produced from two kinds of salmon oil and commercial corn oil. A more detailed description of methyl ester production from salmon oil can be found in the study by El-Mashad et al. (2008). Both salmon oils were obtained from Alaska pink salmon (*Oncorhynchus gorbuscha*) by-products. One salmon oil (acidified) sample was separated from hydrolysate by using a high-speed centrifuge at 7000 rpm for 20 min at relative centrifugal force of 7970 g. The other oil (non-acidified) sample was extracted from fresh salmon by-products by centrifugation. Salmon oil methyl ester was produced using a two-step process. In the first step, salmon oil was esterified with methanol using a molar ratio of methanol to oil of 3.1:1 in the presence of 1% (w/w) H₂SO₄. Then the oil was further transesterified using a 6.2:1 methanol/oil ratio and 1% (w/w) KOH. The reaction times were 60 and 30 min for the first and second steps, respectively. Both steps were performed at 52°C ± 2°C and a mixing rate of 600 rpm. Methyl ester produced from hydrolysate oil was termed acidified, whereas methyl ester produced from fresh salmon by-product oil was termed non-acidified. Methyl ester production from corn oil was carried out in a one-step alkaline-catalyzed transesterification using methanol (20% w/w) and KOH (1% w/w). The reaction was performed at 52°C ± 2°C and a mixing intensity of 600 rpm for 30 min.

FATTY ACID ANALYSIS

Fatty acid contents of non-acidified and acidified salmon oil, non-acidified salmon oil methyl ester, and corn oil methyl ester were measured in duplicate using gas chromatography (GC), as described by Oliveira and Bechtel (2006). About 15 mg of oil or methyl ester was dissolved in 1.9 mL of isooctane (Sigma grade 99%, St. Louis, Mo.). A 100 µL solution of 10 mg mL⁻¹ methyl tricosanoate (Nu-Chek Prep, Inc., Elysian, Minn.) in iso-octane was added, followed by 200 µL of 2N KOH in methanol. The contents were then mixed for 60 s using a vortex mixer. The samples were centrifuged for 3 min at 3400 rpm and the lower layer was discarded. This procedure was repeated twice using 0.5 mL of a saturated solution of ammonium acetate in water, followed by 0.5 mL of deionized water. Methyl esters in isooctane were dried by adding 250 mg of anhydrous sodium sulfate. The samples were then centrifuged at 3400 rpm for 20 min.

The methyl esters were analyzed using a GC (Hewlett Packard 5890, Palo Alto, Cal.) equipped with a flame ionization detector and an auto injector. A 1.0 µL sample was injected and subjected to a 1:100 split ratio. A 100 m capillary column (Supelco 2560) with a 0.25 mm inside diameter and a film thickness of 0.20 microns was used. Hydrogen was used as a carrier gas with a linear flow rate of 27 cm s⁻¹ and a head pressure of 33 psi. Injector and detector temperatures were set at 220°C. The column temperature was held at 75°C for 10 min, increased to 175°C at 20°C min⁻¹ and held for 29 min, and then increased to a final temperature of 225°C at 5°C min⁻¹ and held for 12 min. The detector signal was integrated and quantified using Chrom Perfect Spirit version 5.5 software (Justice Laboratory Software, Denville, N.J.).

RHEOLOGY

A model AR2000 rheometer (TA Instruments, New Castle, Del.) was used to measure the viscosity of each oil or

methyl ester sample. The sample was placed on a Peltier plate, and a 60 mm stainless steel parallel plate was lowered onto it. Before the start of each run, the sample was maintained at the experiment temperature for 10 min to allow for equilibration. The temperatures ranged from 5°C to 40°C. The viscosity was subsequently measured for shear rates ranging from 0.01 to 1000 1/sec.

THERMOGRAVIMETRIC ANALYSIS

A model 2950 thermogravimetric analyzer (TGA) (TA Instruments, New Castle, Del.) was used to measure the thermal stability of the samples. Each sample of 10 mg was heated at 10°C min⁻¹ up to a temperature of 800°C. The sample was maintained in a nitrogen environment with a nitrogen gas flow rate of 40 cm³ min⁻¹.

DIFFERENTIAL SCANNING CALORIMETRY

Crystallization onset temperature was determined by using a model Q1000 differential scanning calorimeter (DSC) (TA Instruments, New Castle, Del.). Sample masses were 1.7 ± 0.22 mg, and hermetically sealed aluminum pans were used for reference and sample. The thermal analytical program was as follows: equilibrate at 40°C, cool at 5°C min⁻¹ to -40°C, and hold isothermally at -40°C for 1 min. Three replicate DSC scans were performed and averaged for each methyl ester sample.

A model 2910 DSC (TA Instruments, New Castle, Del.) was used to measure the thermal properties of the samples. The sample amount used was 2.5 ± 0.3 mg, and each sample was heated from -85°C to 100°C at a rate of 10°C min⁻¹. The sample chamber was purged with nitrogen gas at a flow rate of 75 cm³ min⁻¹.

HEATING VALUE

The heating values of corn and salmon oil methyl esters were determined in duplicate using a model 1241 adiabatic bomb calorimeter (Parr Instrument, Moline, Ill.).

LOW TEMPERATURE PROPERTIES

Manual analysis of cloud point (CP) and pour point (PP) was conducted in accordance with ASTM test methods D2500 and D97, respectively (ASTM, 2003). The apparatus was supplied by Koehler (Bohemia, N.Y.). For both CP and PP analyses, samples were filtered to remove moisture, and approximately 55 mL of sample was used. For CP analyses, samples were examined visually in 1°C intervals, and the temperature where haziness was observed near the bottom of the sample was recorded as the CP. For PP analyses, samples were pulled in 3°C intervals and tipped until movement of the liquid was observed. The lowest temperature where movement could be detected was recorded as the PP.

Automatic analysis of CP and PP were conducted in accordance with ASTM methods D5773 and D5949, respectively (ASTM, 2003). A PSA-70S automatic analyzer (Phase Technologies, Richmond, BC, Canada) was used to measure both CP and PP. Sample volumes were 150 µL and cooling rates were 1.5°C min⁻¹ for each measurement. For both CP and PP measurements, samples were analyzed in 1°C intervals.

Cold filter plugging point (CFPP) was analyzed in accordance with ASTM method D6371 (ASTM, 2003). The apparatus was supplied by Koehler, and methods for preparing,

cooling, and evaluating the sample were similar to those for measurement of CP by ASTM method D2500. At 1°C intervals, a 200 mm water (0.0194 atm) vacuum was applied to draw sample from the test jar into a pipette. The lowest temperature where a 20 mL sample could be drawn into the pipette within 60 s was recorded as the CFPP. For all low temperature properties, three replicate measurements were averaged for each sample.

OIL STABILITY, ACID VALUE, AND SPECIFIC GRAVITY

Oil stability index (OSI) at 60°C ± 0.2°C was measured in accordance with AOCS method Cd 12b-92 (AOCS, 1999). Apparatus was from Omnion Inc. (Rockland, Mass.) under license from Archer Daniels Midland (Decatur, Ill.). Acid value (AV) was measured in accordance with AOCS method Ca 5a-40 (AOCS, 1999). Specific gravity (SG) at 15.6°C was measured in accordance with AOCS method Cc 10c-95 (AOCS, 1999).

RESULTS AND DISCUSSION

FATTY ACID PROFILES

The methyl esters derived from salmon oil and corn oil had very different fatty acid profiles, with salmon oil methyl esters containing more saturated species. The fatty acid profiles of the methyl ester samples are shown in table 1. The corn oil methyl esters consisted predominantly of palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) methyl esters, consistent with the composition of corn oil in other studies (Allen et al., 1999; Ma et al., 1999). In contrast, the salmon oil methyl esters contained a wider range of fatty acids, including myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), linolenic acid (C18:3), eicosapentaenoic acid (C20:5), and docosahexaenoic acid (C22:6) methyl esters. Corn oil and salmon oil methyl esters contained 13.68% (w/w) and 26.64% (w/w) saturated species, respectively.

VISCOSITY

All samples showed Newtonian behavior over the experimental temperature range. This was consistent with results from a previous study (Srivastava and Prasad, 2001), where viscosities of several methyl esters were determined to be Newtonian above 5°C. Table 2 shows the viscosity of oils and methyl esters at different temperatures. As can be seen, each methyl ester had a viscosity value approximately one order of magnitude lower than its corresponding oil. In addition, the viscosities of methyl esters exhibited less dependence on temperature than those of oils. The viscosity of each methyl ester increased by approximately 64% to 77% when temperature decreased from 40°C to 15°C. In comparison, the viscosity of each oil increased by approximately 155% to 159%

Table 1. Fatty acid profiles of oils and methyl esters (g fatty acid/100 g total fatty acid).

	Acidified Salmon Oil	Non-Acidified Salmon Oil	Non-Acidified Salmon Oil Methyl Ester	Corn Oil Methyl Ester
C12:0	0.15	0.14	0.14	0.00
C13:0	0.07	0.07	0.07	0.00
C14:0	7.04	6.85	6.81	0.05
C14:1 cis	0.09	0.10	0.10	0.00
C15:0	0.74	0.88	0.88	0.00
C16:0	14.95	14.83	14.91	11.54
C16:1 trans	0.31	0.33	0.31	0.00
C16:1 cis	6.59	6.13	6.12	0.12
C17:0	0.60	0.68	0.68	0.07
C18:0	3.71	3.12	3.15	2.02
C18:1 trans 9	0.13	0.20	0.18	0.00
C18:1 cis 9&10	19.13	15.53	15.56	28.32
C18:1 cis 11	2.69	2.49	2.51	0.65
C18:2	2.10	2.14	2.14	55.78
C18:3 n6	0.11	0.15	0.12	0.00
C18:3	7.86	11.52	11.47	1.39
C18:2 C9C11	0.00	0.00	0.00	0.02
C18:2 C11C13	2.34	2.74	2.71	0.00
C18:2 all trans	0.00	0.00	0.00	0.01
C20:4	2.79	3.33	3.34	0.00
C22:2 n6	0.11	0.19	0.17	0.00
C20:5	11.52	11.13	11.13	0.01
C22:3 n3	0.22	0.43	0.43	0.00
C22:4 n6	0.12	0.11	0.11	0.00
C22:5 n6	0.25	0.27	0.26	0.00
C22:5	3.42	3.02	3.02	0.00
C22:6	12.96	13.63	13.68	0.01

over the same temperature range. In addition, methyl esters produced from both acidified and non-acidified salmon oils had comparable viscosity values to corn oil methyl ester. The kinematic viscosities at 40°C of methyl esters from corn, acidified, and non-acidified salmon oils were 4.52, 4.97, and 4.52 mm² s⁻¹, respectively.

The dependence of viscosity on temperature could be described by the Andrade equation (Andrade, 1951):

$$\ln(\mu) = A + B/T \quad (1)$$

where μ is viscosity (Pas), T is temperature (K), and A and B are constants. The values of A , B , and the coefficient of determination (R^2) are shown in table 3.

VOLATILITY

Both salmon oil methyl esters had comparable TGA curves to corn oil methyl ester. In addition, the methyl ester samples were more volatile than the oil samples. Figure 1 shows the weight percent of methyl ester and oil samples as

Table 2. Viscosity of oils and methyl esters.

Type of Oil or Methyl Ester	Viscosity (Pas)				
	5°C	10°C	15°C	25°C	40°C
Corn oil	--	--	0.077 ± 0.003	0.051 ± 0.002	0.030 ± 0.000
Acidified salmon oil	--	--	0.074 ± 0.001	0.049 ± 0.001	0.029 ± 0.000
Non-acidified salmon oil	--	--	0.075 ± 0.000	0.052 ± 0.004	0.029 ± 0.000
Corn oil methyl ester	0.0097 ± 0.0003	--	0.0069 ± 0.0002	0.0053 ± 0.0003	0.0039 ± 0.0001
Acidified salmon oil methyl ester	--	0.0094 ± 0.0003	0.0072 ± 0.0003	0.0057 ± 0.0001	0.0044 ± 0.0001
Non-acidified salmon oil methyl ester	0.0099 ± 0.0001	--	0.0070 ± 0.0002	0.0052 ± 0.0001	0.0040 ± 0.0001

Table 3. Parameters of Andrade equation.

Type of Oil or Methyl Ester	A	B	R ²
Corn oil	-14.17	3340.00	0.999
Acidified salmon oil	-14.43	3402.10	0.999
Non-acidified salmon oil	-14.52	3439.50	0.999
Corn oil methyl ester	-12.71	2234.90	0.995
Acidified salmon oil methyl ester	-12.36	2156.10	0.982
Non-acidified salmon oil methyl ester	-12.86	2280.50	0.992

a function of temperature. All three methyl esters had maximum rate of weight loss at 214°C to 217°C. In addition, more than 90% of each methyl ester sample had volatilized at 250°C. In contrast, the oil samples had maximum rate of weight loss at 403°C to 405°C and did not reach 90% weight loss until approximately 440°C. As a comparison, the ignition temperature of diesel fuel ranged from 232°C to 317°C. Sathivel (2005) reported that red salmon and pink salmon oils had decomposition temperatures of 533°C and 668°C, respectively. The author attributed the temperature difference to the presence of impurities such as phospholipids, complexed metals and minerals, and peroxides and their breakdown products.

LOW TEMPERATURE PROPERTIES

The salmon oil methyl esters had slightly higher crystallization onset temperatures than corn oil methyl ester, as shown in table 4. The acidified and non-acidified salmon oil methyl esters had crystallization onset temperatures of -6.7°C and -8.1°C, respectively. In comparison, the corn oil methyl ester had a crystallization onset temperature of -8.8°C. These onset temperature values were determined by performing DSC cooling scans because cooling scans had been shown to correlate better with low temperature properties, such as pour and cold filter plugging points, than heating

Table 4. Low temperature properties of methyl esters.

Parameter	Methyl Esters		
	Corn Oil	Acidified Salmon Oil	Non Acidified Salmon Oil
Crystallization onset (°C)[a]	-8.78 ±0.06	-6.70 ±0.17	-8.10 ±0.10
Cloud point (°C)			
Automatic	--	--	-2.0 ±0.1
Manual	--	--	-2.7 ±0.6
Pour point (°C)			
Automatic	-4.0 ±0.0	-2.7 ±0.6	-3.0 ±0.0
Manual	-4.0 ±1.7	-3.0 ±0.0	-5.0 ±1.7
Cold filter plugging point (°C)	-7.0 ±0.0	-6.3 ±0.6	-7.7 ±0.6

[a] From DSC cooling scans.

scans (Dunn, 1999). Nevertheless, onset temperatures were examined by heating scans as a comparison. The DSC curves for these scans are shown in figure 2. Acidified salmon, non-acidified salmon, and corn oil methyl esters had crystallization onset temperatures of 3.1°C, 3.1°C, and 1.7°C, respectively. These values were higher than those from cooling scans, but they showed the same trend of salmon oil methyl esters having higher onset temperatures. These results were consistent with results from fatty acid analysis: salmon samples had higher concentrations of saturated fatty acid methyl esters, and saturated species had higher melting points than unsaturated ones. As shown in figure 2, the DSC curves for salmon oil methyl esters had much different profiles at lower temperatures than that for corn oil methyl ester. These differences below 0°C are likely due to differences in unsaturated fatty acid methyl ester contents for each sample. Corn oil methyl ester contained mostly oleic (C18:1) and linoleic (C18:2) acid methyl esters as unsaturated species, whereas salmon oil methyl esters contained mostly oleic

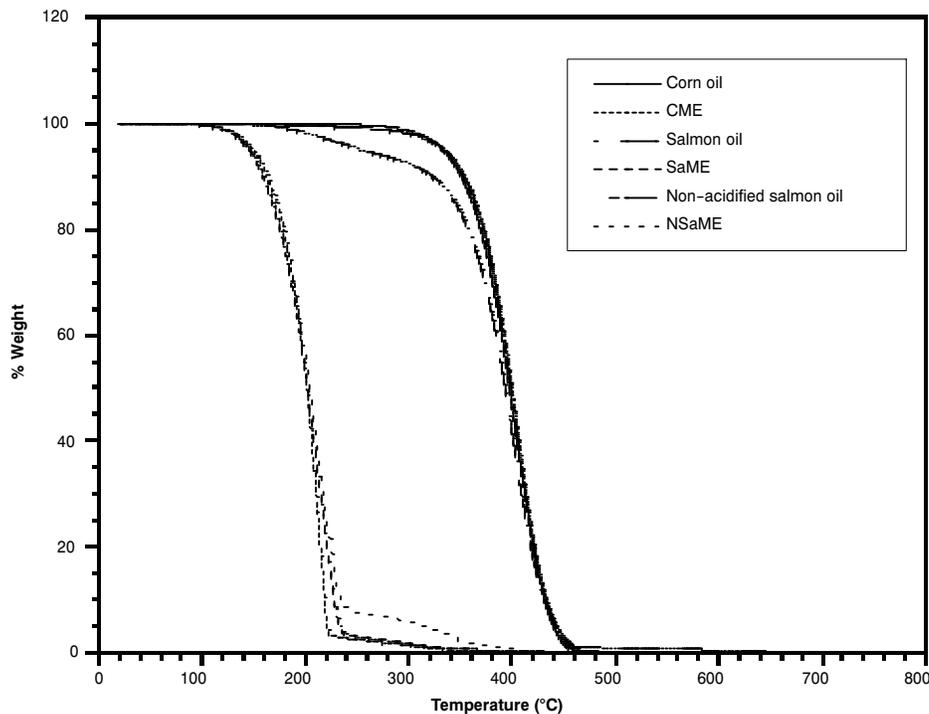


Figure 1. Thermogravimetric analysis (TGA) curves showing weight percent as a function of temperature for acidified salmon, non-acidified salmon, and corn oils as well as their corresponding methyl esters (CME = corn oil methyl ester, SaME = acidified salmon oil methyl ester, and NSaME = non-acidified salmon oil methyl ester).

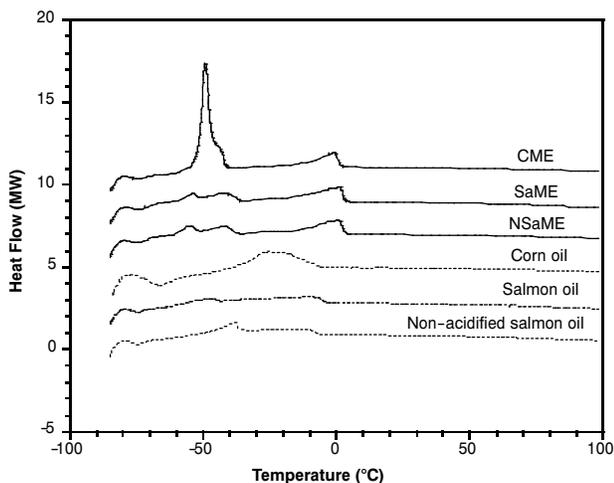


Figure 2. Differential scanning calorimetry (DSC) heating curves showing heat flow as a function of temperature for acidified salmon, non-acidified salmon, and corn oils as well as their corresponding methyl esters. (CME = corn oil methyl ester, SaME = acidified salmon oil methyl ester, and NSaME = non-acidified salmon oil methyl ester).

(C18:1), linolenic (C18:3), eicosapentaenoic (C20:5), and docosahexaenoic (C22:6) acid methyl esters as unsaturated species.

All methyl ester samples had comparable pour point and cold filter plugging point (CFPP) values, as shown in table 4. For pour point measurements, both manual and automatic methods resulted in similar values, which ranged from -3°C to -5°C . These values were comparable to those determined for soybean oil methyl esters (Srivastava and Prasad, 2000; Soriano et al., 2006) and sunflower oil methyl esters (Lang et al., 2001; Soriano et al., 2006), but higher than those determined for rapeseed oil methyl esters (Lang et al., 2001; Soriano et al., 2006). In addition, each methyl ester sample had a CFPP value approximately 3°C to 4°C lower than their corresponding pour point value. It was not possible to determine cloud points for acidified salmon and corn oil methyl esters because they were opaque. However, cloud point values of -2°C (automatic method) and -2.7°C (manual method) could be determined for non-acidified salmon oil methyl ester. These values were comparable to those determined for soybean oil methyl esters (Srivastava and Prasad, 2000; Fukuda et al., 2001; Soriano et al., 2006), rapeseed oil methyl esters (Lang et al., 2001; Soriano et al., 2006), and sunflower oil methyl esters (Srivastava and Prasad, 2000; Fukuda et al., 2001; Lang et al., 2001; Soriano et al., 2006), but much lower than those determined for palm oil methyl esters (Srivastava and Prasad, 2000; Fukuda et al., 2001; Soriano et al., 2006) and tallow oil methyl esters (Srivastava and Prasad, 2000; Fukuda et al., 2001).

OIL STABILITY INDEX, HEATING VALUE, ACID VALUE, AND SPECIFIC GRAVITY

Both salmon oil methyl esters had higher OSI values than corn oil methyl ester, as shown in table 5. In particular, the

acidified salmon oil methyl ester had twice and three times the OSI values of non-acidified salmon oil methyl ester and corn oil methyl ester, respectively. The greater stability of salmon samples might be due to their lower total concentration of unsaturated fatty acid methyl esters. Unsaturated species generally oxidized faster than saturated species. The position of double bonds also had an effect on oxidation rate. For instance, bis-allylic positions were more susceptible than allylic ones to oxidation (Knothe and Dunn, 2003). As shown in table 1, corn oil methyl ester had higher concentrations of fatty acid methyl esters containing allylic positions than salmon oil methyl esters. However, the salmon samples had higher percentages of fatty acid methyl esters containing larger numbers of bis-allylic positions, such as linolenic (C18:3), eicosatetraenoic, (C20:4), eicosapentaenoic (C20:5), and docosahexaenoic (C22:6) acids. Nevertheless, OSI values for salmon samples were comparable to those for commercial soybean oil methyl esters (Dunn, 2005).

The heating values of salmon oil and corn oil methyl esters (table 5) were comparable to those of other methyl esters (Lang et al., 2001; Encinar et al., 2005). Salmon oil methyl ester had a heating value of 38.8 MJ kg^{-1} , which was 13.8% lower than the 45 MJ kg^{-1} value for diesel fuel.

The acid values for the methyl ester samples ranged from 0.149 to $0.211\text{ mg KOH g}^{-1}$, with acidified salmon oil methyl ester having the highest value (table 5). The acid values for the salmon oil methyl esters were comparable to those from other plant oil methyl esters (Lang et al., 2001).

All three methyl ester samples had comparable specific densities at 15.6°C (table 5). These density values were comparable to those for soybean and rapeseed oil methyl esters (Fukuda et al., 2001).

CONCLUSIONS

Salmon oil methyl esters had very different fatty acid profiles compared to corn oil methyl ester. The salmon samples contained 26.64% saturated methyl esters compared to 13.68% for the corn sample. In addition, the salmon oil methyl esters contained relatively large amounts of eicosapentaenoic acid (C20:5) and docosahexaenoic (C22:6) acid methyl esters. Despite these differences, salmon oil methyl esters had comparable viscosity, volatility, low temperature properties, oxidative stability, heating value, acid value, and specific gravity to corn oil methyl esters.

ACKNOWLEDGEMENTS

We thank Allison Dedrick and Rajnesh Narayan for performing some of the rheology, DSC, and TGA experiments. We also thank Professor James Fadel and Scott Taylor of Animal Science Department at UC Davis for help in the analysis of fatty acids. Many thanks also to Professor Bryan Jenkins and Rizaldo Aldas of the Department of Agricultural and Biological Engineering at UC Davis for help in measuring the heating value of biodiesel samples.

Table 5. Oil stability index (OSI), heating value, acid value, and specific gravity of methyl esters.

Methyl Ester	OSI at 60°C (h)	Heating Value (MJ kg^{-1})	Acid Value (mg KOH g^{-1} oil)	Specific Gravity at 15.6°C
Corn oil	3.65 ± 0.18	38.7 ± 0.2	0.165 ± 0.011	0.893
Acidified salmon oil	12.15 ± 0.25		0.211 ± 0.012	0.885
Non-acidified salmon oil	6.12 ± 0.31	38.8 ± 0.3	0.149 ± 0.027	0.891

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