Determining the Influence of Contaminants on Biodiesel Properties

Final report prepared for:

The Iowa Soybean Promotion Board

Submitted by:

Jon H. Van Gerpen
Earl G. Hammond
Lawrence A. Johnson
Stephen J. Marley
Liangping Yu
Inmok Lee
Abdul Monyem

Iowa State University

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1. Introduction

Biodiesel, as used in this report, is defined to be the alkyl monoesters of vegetable oils or animal fats. It is produced by chemically reacting the oil or fat with an alcohol in the presence of a catalyst. The products of this reaction are the monoesters, known as biodiesel, and glycerin, which is a high value byproduct. Because of its low cost, methanol is the most common alcohol used but ethanol is used in some cases. In Europe, rapeseed oil is the most common vegetable oil feedstock and in the United States, soybean oil is generally used. All of the work presented in this report uses biodiesel produced from methanol and soybean oil. This particular form of biodiesel is often called *methyl soyate*.

The American Society for Testing and Materials (ASTM) and the American Society of Agricultural Engineers (ASAE) are currently developing specifications for biodiesel. Although these specifications are still in draft form, they will almost certainly contain limits on the percent esterification, the cloud point, the pour point, the amount of free glycerin and total glycerin, as well as many other properties. The limits on these properties are set as compromises between the desire of the engine manufacturer to have no contaminants and the desire of the fuel supplier to have levels that will be easy and inexpensive to meet. It would be very useful to have some data to guide the standards setting process. Biodiesel standards that are too strict will increase the cost of the fuel and standards that are too lax will increase the likelihood that consumers will encounter fuel-related problems. Both possibilities could impact the market acceptance of biodiesel. The problem directly impacts biodiesel cost since the lack of knowledge about contaminant effects will probably result in very conservative standards that will be expensive to satisfy.

The amount of data on biodiesel properties and its effect on diesel engine performance and emissions has greatly increased over the past three years, due in large part to the financial support of soybean farmers through their funding of the National Biodiesel Board. A serious problem has now developed because it has been found that different researchers, running the same standardized tests on biodiesel, have obtained very different results. For example, until recently, the cetane number of methyl soyate was believed to be about 48. Now data has been presented that the cetane number may actually be as high as 60. This change is well beyond measurement error and is probably attributable to fuel contamination. Thus, in addition to the need to control fuel quality to protect the engine, there is also the need to understand the general relationship between contaminants and fuel properties.

The objective of this project was to develop the database that would provide a basis for setting realistic specification values for biodiesel. This report will discuss each of the expected contaminants in terms of their impact on engine life and performance and present data to justify a particular specification value. The contaminants to be considered include water, free glycerin, bound glycerin, alcohol, free fatty acids, soaps, and residual catalyst. During the reporting year, unsaponifiable matter, microorganisms, and oxidation products were added to the list of important contaminants, and the effects of their presence on the properties of biodiesel were also studied.

This project was jointly funded by three entities: the Iowa Soybean Promotion Board, the Iowa Energy Center, and the Iowa Department of Agriculture's Renewable Fuels Committee. Due to a delay in receiving the funds from the Iowa Department of Agriculture, a portion of the project has not been completed. Rather than request an extension of the overall project, we are reporting on the results of the project to date and not including the portion of the project yet to be

completed The remaining work consists primarily of engine testing of the impact of monoglycerides on injector deposits. A second report will be submitted to all funding agencies when this work is complete. We expect this report to be finalized by September 30, 1996.

2. Background

This section presents the specification values for diesel fuel and biodiesel. The American Society for Testing and Materials maintains the standard for petroleum-based diesel fuels. It covers three grades of diesel fuel although No. 2-D is the most common grade used in trucks and agricultural equipment. No 1-D is used as a blending agent during the winter to improve diesel fuel cold flow properties.

2.1 ASTM Specifications for Diesel Fuel Oils (D975)

ASTM standard D975 covers three grades of diesel fuel oils.

1. **Grade No. 1-D**: A light distillate fuel for applications requiring a higher volatility fuel for rapidly fluctuating loads and speeds as in light trucks and buses. The specification for this grade of diesel fuel overlaps with kerosene and jet fuel and all three are commonly produced from the same base stock. One major use for No. 1-D diesel fuel is to blend with No. 2-D during winter to provide improved cold flow properties.

2. **Grade No. 2-D**: A middle distillate fuel for applications that do not require a high volatility fuel. Typical applications are high speed engines that operate for sustained periods at high load.

3. **Grade No. 4-D**: A heavy distillate fuel that is viscous and may require fuel heating for proper atomization of the fuel. It is used primarily in low and medium speed engines.

ASTM D975 specifies the property values shown in Table 1 for these three grades of diesel fuel.

2.2 National Biodiesel Board Specifications for Biodiesel

The National Biodiesel Board (NBB) maintains a specification for biodiesel that is currently under review for acceptance as a standard by the ASTM. The NBB maintains a list of biodiesel fuel suppliers who have agreed to supply fuel meeting these specifications. The specification values are shown in Table 2. The values given in the table were adopted based on European experience with rapeseed methyl esters. It is not known whether the values given are appropriate for biodiesel produced and consumed in the United States. The objective of this project is to investigate whether the specifications associated with contaminants are valid.

Table 1. ASTM D 975 Specifications for Diesel Fuel

Property	ASTM test	Grade 1-D	Grade 2-D	Grade 4-D
Flash Point (°C)	D93	38	52	55
Water and Sediment (%)	D1796	0.05	0.05	0.50
Distillation Temperature	D86			
90% recovered, min		none	282	none
max		288	338	none
Kinematic Viscosity	D445			
min		1.3	1.9	5.5
max		2.4	4.1	24.0
Ramsbottom carbon residue on 10% distillation residue	D524	0.15	0.35	none
Ash, % mass, max	D482	0.01	0.01	0.10
Sulfur, % mass, max	D129	0.50	0.50	2.00
Copper Strip corrosion rating, max 3 hr at 50°C	D130	No. 3	No. 3	none
Cetane Number, min	D613	40	40	30
Cloud point, °C, max	D2500	Guidance only. Should be 6°C higher than the tenth percentile minimum ambient temperature for the region		

For Iowa,

Month	10th % minimum temp
Oct	-2°C
Nov	-13
Dec	-23
Jan	-26
Feb	-22
Mar	-16

Table 2. The National Biodiesel Board Specifications for Biodiesel

Property	ASTM Method	Limits	Units
Flash Point	D 93	100 min	°C
Water and Sediment	D 1796	0.050 max	Vol. %
Carbon Residue, 100% sample	D 4530	0.050 max	wt %
Sulfated Ash	D 874	0.020 max	wt %
Kinematic Viscosity, 40°C	D 445	1.9 - 6.0	mm^2/sec
Sulfur	D 2622	0.05 max	wt %
Cetane Number	D 613	40 min	
Cloud Point	D 2500	By customer	°C
Copper Strip Corrosion	D 130	No. 3b max	
Acid Number	D 664	0.80 max	mg KOH/g
Free Glycerin	GC	0.020 max	wt %
Total Glycerin	GC	0.240 max	wt %

3. Results

This section presents the results of investigation into the correct values of contaminant concentrations in biodiesel to be used as an engine fuel. The contaminants considered are: unsaponifiable matter, water free glycerin, bound glycerin, alcohol, free fatty acids, soaps, residual catalyst, and oxidation products.

3.1 Unsaponifiable matter

Typical crude soybean oil contains as much as 1 6% *unsaponifiable matter*, which consists of plant sterols, tocopherols and hydrocarbons as major components, with very small quantities of pigments and minerals. The unsaponifiable matter is not affected by ester preparation, so it is likely to be present in similar amounts in biodiesel to its level in crude soybean oil. The compounds in the unsaponifiable matter have varying amounts of unsaturation, and include open

chain, cyclic aliphatic and aromatic functional groups which are similar to many of the compounds present in diesel fuel The unsaponifiable matter may have no harmful effects on engine performance except possibly for a change in the crystallization onset temperature caused by polar sterols. Therefore, the effect of the unsaponifiable matter on crystallization properties of neat esters and 20% esters/80% diesel fuel blends were studied. A second issue related the unsaponifiable matter is whether it has any impact on the cetane number of the biodiesel. This was investigated also.

3.1.1 Crystallization Onset Temperature

Simulated unsaponifiable matter that resembles that found in crude soybean oil was formulated with 70% β-Sitosterol, 14% α-tocopherol, 8% Squalane, and 8% Squalene. Distilled methyl soyate (purchased from Interchem Environmental of Kansas City, MO) was prepared with various unsaponifiable levels (0%, 1%, 2%, and 3%), and 20% (by volume) blends of methyl soyate and No. 1 diesel fuel were prepared with the same concentrations of unsaponifiables. Two separate samples were prepared for each unsaponifiable level, and two measurements were made for each sample. Unsaponifiable matter was measured by the AOCS Official Method Ca 6a-40. Crystallization properties were characterized by the cloud point (ASTM Standard Method D2500), pour point (ASTM Standard Method D97) and the crystallization onset temperature (Tco) determined by differential scanning calorimeter (DSC) analysis. The Tco was determined as the end point (where the tangent of the peak meets the baseline) of the highest melting transition peak, that is, the lowest temperature at which the sample was completely liquid.

The content of unsaponifiable matter in various commercial and experimental methyl soyates and the soybean oils from which they are derived were determined and are shown in Table 3. Replications agreed well, and the Interchem methyl soyate, which was double-distilled, contained practically no unsaponifiable matter. When 1% of the simulated unsaponifiable matter was added to the Interchem methyl soyate, the sample was analyzed as having 0.91%

Table 3. Unsaponifiable matter content of various methyl esters and soybean oils

Samples	Unsaponifiable Matter (%)		
	Rep. 1	Rep. 2	Average
Methyl esters (purchased from Interchem)	0.06	0.00	0.03
Methyl esters (Interchem) with 1% unsaponifiables added	0.91	0.90	0.91
Methyl esters (Purchased from Calgene)	0.50	0.48	0.49
Crude soybean oil (West Central Coop.)	0.41	0.46	0.44
Methyl esters made from West Central soybean oil	0.49	0.51	0 50
Commercial RBD soybean oil	0.48	0.43	0 46
Methyl esters made from commercial RBD soybean oil	0.44	0.52	0.48

Table 4. The effect of unsaponifiable matter on crystallization properties of neat methyl esters and 20% esters-kerosene blends

Samples	% Unsaps	Tco (°C)	Cloud Point (°C)	Pour Point (°C)
Neat esters	0% (control)	4.7[a]	-5	-3
	1%	3.8[b]	-5	-3
	2%	3.8[b]	-5	-3
	3%	19 1[c]	8	-3
20% esters blends	0% (control)	-14.7[d]	-28	<-36
	1%	-14.9[d]	-28	<-36
	2%	-14.2[d]	-28	<-36
	3%	-14.6[d]	-28	<-36

Values with different superscript in the same row are significantly different at $\alpha=0.05$

unsaponifiable matter, which indicated that 91% of the added unsaponifiable matters were recovered by the extraction procedure of the analysis method. The amounts of unsaponifiable matter measured in several samples of soybean oil and the methyl esters produced from those samples were all between 0.4 to 0.5%, which is on the low end of the expected range. The amounts of unsaponifiable matter in both crude and RBD oils, were not significantly changed (at $\alpha=0.05$) during the preparation, transesterification, and washing of the methyl soyates.

The effect of unsaponifiable matter on the crystallization properties of biodiesel fuels is shown in Table 4. Unsaponifiable matter up to 2% caused no adverse effect on Tco in the neat esters The Tco of ester samples containing small amounts (<2%) of unsaponifiables was even slightly less than the neat esters because of a melting point depression effect of such "impurities." At the 3% unsaponifiable matter level in neat esters, the Tco was significantly increased; however, in the 20% esters/No. 1 diesel fuel blends, there was no significant effect on Tco up to 3% unsaponifiable matter. Results of cloud and pour point measurements agreed with those from DSC Only the sample with 3% unsaponifiables had a higher cloud and pour points than the others when measured in neat esters, and this sample showed no difference in 20% blends with diesel fuel. It appears that at normally expected levels of unsaponifiable matter, there should be no deleterious effect on the cold flow properties.

3.1.2 Cetane Number

One major difference between different samples of biodiesel may be whether the fuel contained the unsaponifiable matter that was originally present in the soybean oil. Most of the soybean-based biodiesel used during 1993 and 1994 in the United States was processed using a distillation process that removed the unsaponifiable matter along with the glycerin and all other materials with higher boiling temperatures than the esters themselves. This fuel, commonly identified as "double distilled fuel," may have different properties than undistilled fuel due to the removal of the unsaponifiable matter.

To investigate the effect of unsaponifiable matter on the cetane number, the same synthetic unsaponifiable material described above was blended with #2 diesel fuel at 1% and 2% levels (by

Table 5. Effect of Unsaponifiable Matter on Cetane Number.

Fuel	Cetane Number (ASTM D613)
Diesel Fuel	45.9
1% Unsap in Diesel Fuel	44.2
2% Unsap in Diesel Fuel	45.6
Methyl Soyate	52 7
1% Unsap in Methyl Soyate	53.7
2% Unsap in Methyl Soyate	53 4
3% Unsap in Methyl Soyate	52 8

weight) It was not possible to keep more than 2% of this material in solution with diesel fuel. The unsaponifiable matter was blended with methyl soyate at 1%, 2%, and 3% levels.

Table 5 shows the effect of unsaponifiable matter on the cetane number of diesel fuel and methyl soyate The data show that the presence of unsaponifiable matter in the fuel has no effect on the cetane number of the fuel, either for diesel fuel or for methyl soyate. The slight changes in cetane number are generally less than the 0.7-0 8 ASTM repeatability specification for cetane number and are always less than the 2.6-3.1 reproducibility specification.

The currently proposed standards for biodiesel do not contain a specification for unsaponifiable matter. The data presented here do not indicate that such a specification is necessary.

3.2 Water

Water is a major source of fuel contamination. While fuel leaving a production facility may be virtually water-free, once it enters the existing distribution and storage network, it will come into contact with water. Water typically enters fuel tanks through vents and seals as humidity in the air. The water condenses or is dissolved into the fuel. Virtually all diesel fuel storage tanks can be assumed to contain some water. In some cases, water is even used for tank cleaning and as ballast [1].

Water in the fuel generally causes two problems. First, it can cause corrosion of engine fuel system components. The most direct form of corrosion is rust, but water can become acidic with time and the resulting acid corrosion can attack fuel storage tanks.

The other major problem associated with water contamination is that it contributes to microbial growth. There are species of yeast, fungi, and bacteria that will grow at the interface between the fuel and any free water that has collected at the bottom of a storage tank. The organisms produce sludges and slimes that can cause filter plugging [1]. Some of the organisms can convert the sulfur in the fuel to sulfuric acid which can corrode metal fuel tanks.

Water will be present in the fuel in two basic forms. It will be dissolved in the fuel or as free water collected at the bottom of the sample. The amount of water dissolved in the fuel depends on its solubility. The amount of free water in the sample depends primarily on the quality of the "housekeeping" practices followed by the fuel distributors and consumers. Frequently draining the water from storage tanks, ensuring that vents and seals do not allow rainwater to

enter, and not drawing from the bottom of the tank, should prevent large amounts of free water from entering the system.

To investigate the problem of water contamination, the solubility of water in methyl esters was measured and compared with diesel fuel. Then, since the primary problem associated with free water is microbial growth, the tendency of the methyl esters to support microbial growth was investigated

3.2.1 Water Solubility

The objectives of our study were to compare the solubility of water in methyl esters, 20% esters/80% No. 2 diesel fuel blends, and No. 2 diesel fuel, and to determine the effect of oxidation level on the solubility of water in methyl esters. Fuel oxidation was included as a variable since the polar nature of the hydroperoxides formed during oxidation might cause the oxidized fuel to have different solubility properties than the unoxidized fuel.

Figure 1 shows the solubility of water in diesel fuel. This plot was constructed from a figure that used different units (ml/gal) in reference [2]. At 25°C, the solubility of water in diesel fuel is only about 60 ppm. This is quite low and probably results in no adverse effects. ASTM standard D975 for No. 2 diesel fuel allows up to 0.05% water (500 ppm) which includes both dissolved and free water. With No. 2 diesel fuel, the specification assumes that most of the water
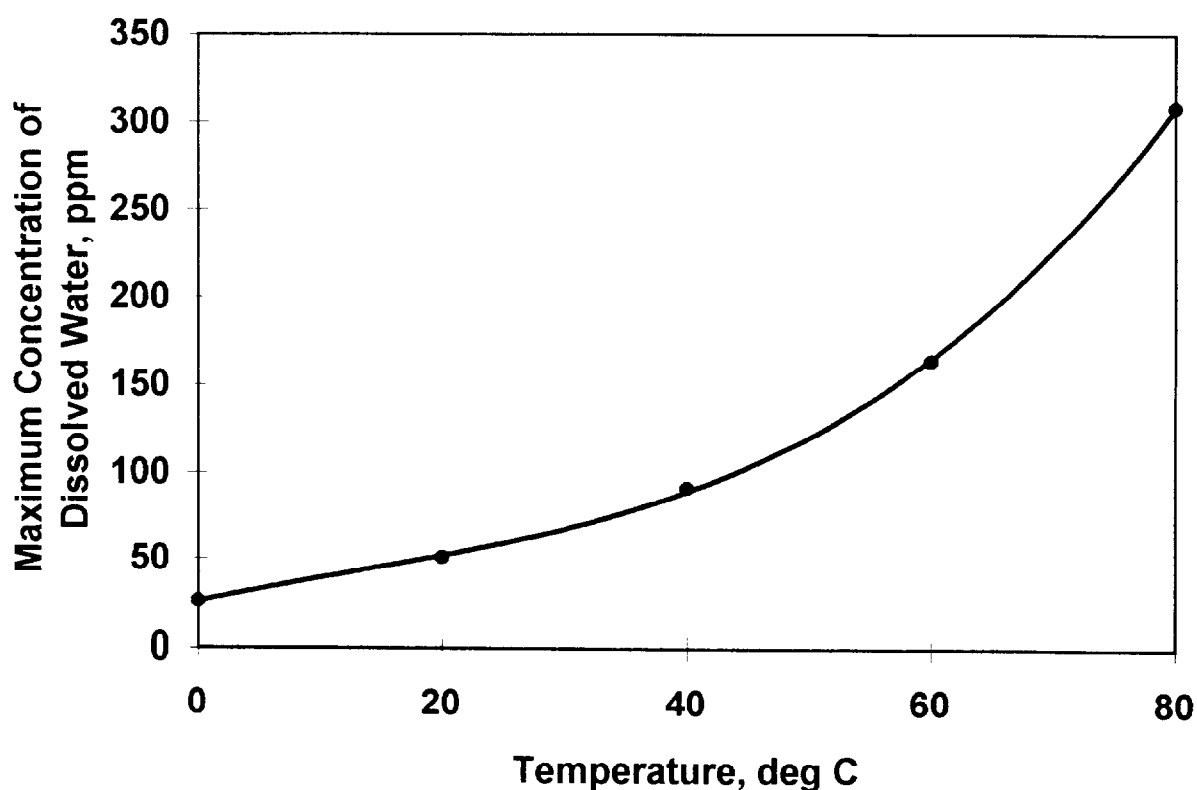

Figure 1. Solubility of water in diesel fuel. (Based on [2])

is present as free water. The tests conducted as part of this study compared the solubility of water in methyl esters with its solubility in diesel fuel

The fuels (highly-oxidized Interchem methyl esters with a peroxide value (PV) =124, freshly made methyl esters with a PV=20, 20% blend of methyl ester in 80% No. 2 diesel fuel, and No. 2 diesel fuel) were vigorously mixed with an equal volume of water. The water content in the fuels was then determined by Karl Fischer titration after 0, 1, 3, 18, 24, and 120 hours of mixing.

Table 6 summarizes the results from the water solubility tests The data shown for zero hours was the baseline case before water was added. Karl Fischer (K-F) titration values were zero or negative when samples of highly-oxidized Interchem methyl soyate were titrated This result may have been caused by re-oxidation of the iodine ion, formed during blank titration of the solvent methanol, by the peroxides in the highly oxidized methyl soyate (PV=124). Slight signs of the re-oxidation were observed during the titration of freshly-prepared methyl soyate (PV=20), however, positive titrations were obtained with this sample. The freshly-prepared esters, vacuum-dried after washing with water, had a fairly low water content of about 40 ppm, which was almost as low as that of No. 2 diesel fuel When mixed vigorously with water, the methyl soyate was saturated with water in one hour, and the water level in the esters did not increase after 5 days of mixing. There was no change in the peroxide value of the esters samples during the mixing. The saturation level of water in methyl soyate was about 40 times greater than that of diesel fuel and was close to the 1500 ppm level, which was observed for water-washed esters before vacuum drying. The diesel fuel had a very low water content at the start of the test, about 28 ppm, and no increase was observed after 18 hr of vigorous mixing The differences between the values reported for the solubility, 28 ppm, 81 ppm, and 30 ppm, are not considered to be statistically significant. Their average is close to the value reported in Figure 1. The 20% blend of methyl soyate in 80% diesel fuel had a water content similar to diesel fuel, and there was no increase in water content after an extended period of vigorous mixing. These results suggest the possible separation of free water when water-saturated esters are blended with diesel fuel.

Table 6. Water contents (ppm) in various methyl soyates, No. 2 diesel fuel and 20% esters-diesel fuel blend after vigorous mixing with water for various periods at 25°C.*

	0 HR	1 HR	3 HR	18 HR	1 D	5 D
Methyl soyate (PV=124, Interchem)	0	0	0	0	-	-
Methyl soyate (PV=20)	37[c]	1460[a]	1595[a]	-	1255[b]	1225[b]
20% Blend (PV=20)	40[a]	37[a]	38[a]	-	33[a]	45[a]
Diesel fuel (No.2)	28[a]	-	81[a]	30[a]	-	-

*The values are the average of duplicate measurements.
Values with different superscript in the same row are significantly different at $\alpha=0.05$

3.2.2 Microorganisms

One advantage of biodiesel, over petroleum-based diesel fuel, is its biodegradability, however, this biodegradability may allow biodiesel to be more susceptible to microbial contamination than petroleum fuels. Our study was focused on comparing the bioavailibility of biodiesel to diesel fuel for different types of microorganisms.

Dirt samples were taken from a methyl soyate storage site and were inoculated into a liquid media containing various nitrogen sources (Yeast N-base and Pseudomonas basal mineral N-base) and carbon sources (No. 2 diesel fuel and methyl soyate fuels) and the microbial growth was compared The culture media were incubated on a shaker in the dark at room temperature, and their growth was monitored by measuring the optical density of the aqueous phase of the culture media with a spectrophotometer. To isolate the microorganisms from the various cultures, aliquots of the culture media were spread on agar plates (Yeast nitrogen base with 0.5% glucose and Pseudomonas basal mineral medium with 1.4% glucose) and incubated at room temperature. The single colonies were further streaked on additional agar plates until pure cultures were obtained. The morphology of the isolated microorganisms was observed.

The growth of microorganisms on the various liquid media are shown in Table 7. The yeast N-base with 1% methyl ester media had no significant increase in turbidity until 6 days incubation, but at 6 days, visible mold was found in the liquid medium although the solution was still clear. Significant growth was found after 3 days incubation in the yeast N-base with 1% diesel fuel. Pseudomonas N-base with 1% methyl ester showed growth after 2 days incubation, while Pseudomonas N-base with 1% diesel fuel showed growth after 3 days incubation. The same trend of growth was also found on the agar plates. However, cultures from diesel and methyl ester containing liquid media indicated different types of microbial growth on the agar plate. Two cultures with different morphologies were isolated from the yeast N-base diesel medium on the yeast N-base agar plates. Four cultures were isolated from the yeast N-base methyl ester medium on the yeast N-base agar plates Of these, two had the same morphology as those isolated from the diesel fuel medium and one of the remaining isolates was mold. On Pseudomonas N-base agar plates, two cultures were isolated from the Pseudomonas N-base diesel fuel medium and four from the Pseudomonas N-base methyl ester medium. Of these, one appeared to be the same as that isolated from the diesel fuel medium and two were molds. No molds were observed or isolated from any of the media with diesel fuel as the carbon source, therefore, at this point, it seems that methyl ester is more hospitable to molds than diesel fuel. Further studies are needed to identify the isolated cultures, and to find the storage conditions for biodiesel that will prevent microbial growth.

Table 7. The growth of microorganisms on various diesel and methyl soyate liquid media*

Liquid media	Absorptance			
	1 day	2 days	3 days	6 days
Yeast N + methyl ester	0 016	0.023	0.030	0.009
Yeast N + diesel	0.042	0.067	0.250	0.448
Pseu N + methyl ester	0.057	2.004	4.700	10.24
Pseu N + diesel	0.038	0.073	0.177	0.147

*The data reported in the table are the optical density measured by the spectrophotometer at 520 nm

The conclusion of this study of water contamination is that water is a much more significant issue for biodiesel than for diesel fuel. Methyl soyate can contain up to 40 times more dissolved water than diesel fuel. In fact, if methyl soyate comes into contact with free water during transport and storage, which is a virtual certainty, it will absorb 1200-1500 ppm of this water. This is well above the 500 ppm level allowed by the current specification. The greater solubility of water in methyl soyate may require that the acceptable level of water be raised to 1500 ppm. The fact that this additional water is bound to the fuel and not available for microbial growth may justify this increase. The increase in the activity of microorganisms on biodiesel, especially molds, is also reason for concern. In applications where the fuel is expected to come into contact with sufficient quantities of free water to support the growth of microorganisms, the fuel should be treated with a biocide to prevent their growth. This will have an adverse effect on the biodegradability of the fuel.

3.3 Free Glycerin

Free glycerin, a product from the transesterification reactions, is not present when esters are washed thoroughly with water. However, glycerin may be present in biodiesel as a result of inappropriate processing, such as insufficient separation of the glycerin phase or insufficient washing by water after separation. Glycerin will also be present when distillation is used as the primary mechanism for its removal. Free glycerin in the fuel is suspected to contribute to deposit formation in the engine. Since its solubility in methyl esters is low, free glycerin tends to collect, over time, at the bottom of storage tanks. Free glycerin also has a high affinity for water and monoglycerides. If it is present, it is suspected that it will selectively attract these compounds from the ester and concentrate them at the bottom of the tank. The objectives of this study were to determine the solubility of glycerin in methyl esters and the effect of water on the solubility of glycerin in methyl esters.

Double-distilled methyl esters purchased from Interchem (Kansas City, MO) were mixed with pure glycerin (Fisher Scientific, Fair Lawn, NJ), and the free glycerin content was measured by the AOCS Official Method Cc 17-79 after 4 hr, 24 hr, and 48 hr of vigorous mixing. To determine the effect of water on the solubility of glycerin in methyl esters, double-distilled methyl esters were mixed with a 1:1 (V/V) mixture of pure glycerin and distilled water. The free glycerol content in the ester-phase was measured by the AOCS Official Method Cc 17-79 after 4 hr, 8 hr, 48 hr, and 72 hr of vigorous mixing. After 72 hr of mixing, the ester-phase was separated from the lower phase and dried on a rotary evaporator to remove the water, and the free glycerin in the dried ester was measured. To determine the effect of the presence of water saturation on the solubility of glycerin in methyl esters, methyl esters were first saturated with water, then mixed with pure glycerin for 48 hr, and the free glycerin content was measured.

The results are presented in Tables 8 and 9. The solubility of glycerin in methyl esters was quite low. Even after 48 hr of mixing, the free glycerin content was less than 0.15%. In the presence of a large amount of water, the solubility of glycerin in the methyl esters was zero. This is because glycerol is more easily dissolved in water than ester. Extending this result to the processing of esters, we can assume that during the preparation of methyl esters, the washing step will wash out all of the free glycerin, if any is present in the ester-phase. When the ester is

Table 8. The solubility of glycerin in methyl esters

Mixing time	4 hr	24 hr	48 hr
% free glycerin in ester	0.072	0.082	0.144

Table 9. The effect of water on the solubility of glycerin in methyl esters

	In the presence of a large amount of water					Saturated with small amount of water first
Samples	4 hr mixing with water present	8 hr mixing with water present	48 hr mixing with water present	72 hr mixing with water present	72 hr mixing with water removed	48 hr mixing
% free glycerin	0	0	0	0	0	0.187

previously saturated with water, but no separate water phase is present, the solubility of glycerin in esters may be increased slightly, over that of dry esters from 0.144% to 0.187%.

The free glycerin level allowed by the current specification is 0.02%. This is well below the solubility of glycerin in the ester but is easily achieved by water washing. Since the primary problems associated with glycerin are due to its existence as a separate phase, it would appear that this specification could be relaxed somewhat. A level of 0.05% would probably be acceptable and might allow alternatives to water washing. Idaho Transtech, Inc. has proposed using glycerin itself as the washing fluid which would result in higher levels of dissolved glycerin in the final biodiesel product [3].

3.4 Bound Glycerin

Figure 2 shows the relative amounts of monoglycerides (MG), diglycerides (DG), and triglycerides (TG) that may exist as the amount of alcohol is varied during transesterification [4]. Collectively, these compounds will be referred to as *bound glycerin*. Although the data shown are for sunflower oil and ethanol, the amounts should be similar for soybean oil and methanol. When 100% excess alcohol is used (2.0 times theoretical) the amount of unreacted TGs should be essentially zero. However, some MG and DG may be present. Due to their polarity, these partially reacted glycerides should be preferentially attracted to the glycerin phase and removed when the phases are separated. Their actual concentration in the ester may be less than shown in the figure.

There are two reasons for keeping the amount of bound glycerin low. First, if the bound glycerin is in solution and is injected into the engine, there is suspicion that it will cause carbon deposits on the fuel injector tips and piston rings. This suspicion is apparently based on historical
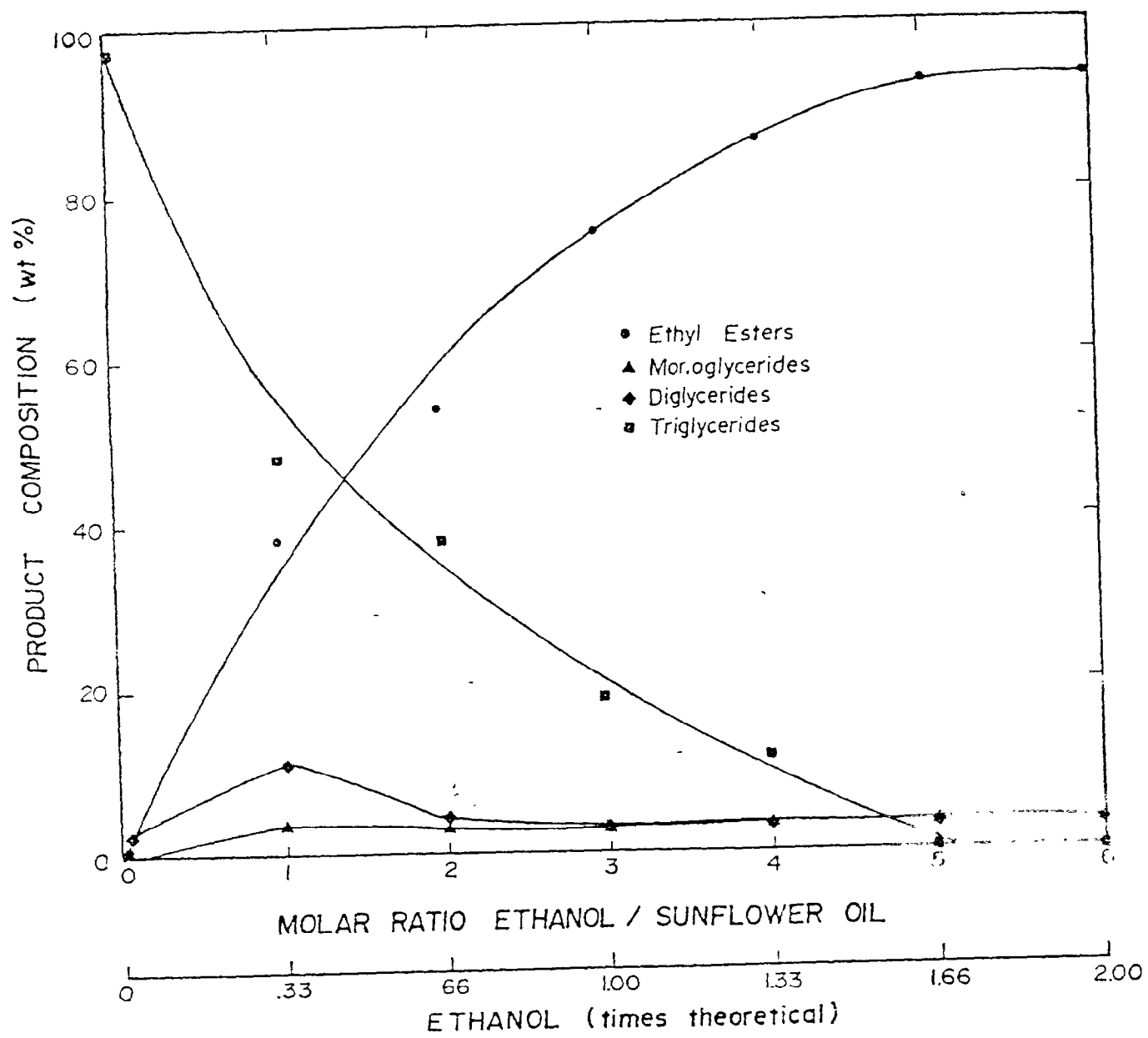
Figure 2. Effect of alcohol/triglyceride ratio on transesterification product composition. [4]

experience with trying to use vegetable oils as diesel fuel extenders in the 1970s [5]. The current specification level of bound glycerin in the NBB specification is 0.24%. This translates, for soybean oil, into 0.92% MG if all the bound glycerin is present as MG, and 1.59% DG if all the bound glycerin is present as DG. The second reason for controlling the amount of bound glycerin is that the partially reacted glycerides, particularly the saturated MGs, have very low solubility in methyl esters and require high temperatures to keep them from crystallizing.

Table 10 shows the analysis of a creamy paste-like material that was extracted from a plugged fuel filter that had been used with a mixture of 20% biodiesel in 80% No. 1 diesel fuel. The plugged fuel filter was a final filter located immediately before the fuel fill nozzle at a bus refueling station in Ames, Iowa. The material was provided by Fosseen Manufacturing and Development, the manager of the biodiesel demonstration project.

The paste consisted of about 25% MGs and DGs. The MGs are over 95% saturated, as would be expected since the saturated MGs crystallize at higher temperatures than the unsaturated MGs. There were two surprising results associated with this analysis. First, that the DGs were present in almost as large an amount as the MGs. This was unexpected since the DGs have lower crystallization temperatures than the MGs. Second, that the DG composition is not skewed toward the saturated species but rather approximates the fatty acid composition of the original soybean oil. At the start of this project it was expected that the principal compounds to be involved with crystallization would be saturated MGs. However, based on the data presented in Table 10, the study was expanded to include DGs, and potential interactions between MGs and DGs

The problem of bound glycerin was addressed by this project in two ways: engine testing to determine whether the bound glycerin in solution would contribute to engine injector tip deposits, and laboratory testing to identify the crystallization characteristics of the MGs and DGs. We did not include TGs in the crystallization study because the MGs and DGs should crystallize sooner. The engine testing portion of this project has not been completed. This is the portion of the project that was dependent on a delay in funding The results of this work will be reported at a later date. This portion of the study focused on the effects of selected pure MGs and DGs on the crystallization properties of methyl esters, the effect of incomplete transesterification on the amount and composition of MGs and DGs in methyl soyate, and the effect of mixtures of MGs and DGs on the Tco of methyl soyate.

3.4.1 Crystallization of Pure Monoglycerides and Diglycerides

Monoglycerides and diglycerides should be present in methyl soyate in very small amounts when transesterification is properly executed. However, as noted above, both MGs and DGs were found in large amounts on plugged fuel filters Due to their high melting points and polar

Table 10. Composition of material removed from plugged fuel filter.

		Fatty acid composition				
Isolates	% in Fuel Filter Sample	16:0	18:0	18:1	18:2	18:3
MG	14%	60.8%	35.9%	1.7%	1.37%	0.15%
DG	11%	14.9%	6.3%	29.6%	44.5%	4.68%

characteristics, MGs and DGs, especially saturated MGs and DGs, may cause crystallization problems in fuels.

Samples of pure MGs and DGs in methyl ester were prepared by dissolving three MGs (monopalmitin, monostearin, and monoolein) and one DG (dipalmitin) in heated methyl esters. The esters were Interchem double distilled esters that had been found to have very low levels of MG and DG.

The effects of the pure MGs and DG on the crystallization of methyl esters are presented in Table 11. In addition to the crystallization onset temperature, T_{CO}, measured by Differential Scanning Calorimetry (DSC), the pour point (PP), measured by ASTM standard method D97, and the cloud point (CP), measured by ASTM standard method D2500, were measured also. The values in the table are the average of duplicate measurements. The presence of the MGs and DG, up to 1%, did not change the pour point of the esters samples. However, the cloud point of the samples increased with increasing amounts of the saturated MGs or DG, and even the sample with 0.05% saturated MG had a CP significantly higher than that of the control. Dipalmitin had a similar effect on the crystallization properties of the methyl esters as was found for monopalmitin. This was surprising because the DGs generally have higher crystallization temperatures. Monoolein (up to 2%), as expected, had no effect on the CP and PP of the esters. This confirmed observations made about the fatty acid composition of MGs that were collected from the plugged fuel filter. About 95% of the MGs in the plugged filter sample were saturated MGs. Unsaturated MGs seemed not to contribute to the crystallization of the fuels at low temperature.

Determination of T_{CO} from DSC thermograms was difficult (thus, not very reproducible) for the samples with less than 0.5% MG because the melting-transition peaks of the MGs were very flat on the baseline in this range. This was especially true for monostearin. For the same

Table 11. Crystallization properties of neat methyl esters with various amounts of pure mono- or di-glyceride.

% MG or DG in esters	1-monopalmitin			1-monostearin			monoolein		dipalmitin	
	T_{co} (°C)	CP (°C)	PP (°C)	T_{co} (°C)	CP (°C)	PP (°C)	CP (°C)	PP (°C)	CP (°C)	PP (°C)
2.0	57.5	-	-	56.5	-	-	-6	-3	29	0
1.0	47.4	22	-3	51.6	26	-3	-6	-3	21	-3
0.75	-	19	-3	-	26	-3	-	-	-	-
0.6	-	-	-	-	-	-3	-6	-3	14	-3
0.5	17.7	10	-3	55.3	22	-3	-	-	11	-3
0.4	15.0	8	-3	54.8	11	-3	-6	-3	5	-3
0.3	11.7	1	-3	50.0	9	-3	-	-	1	-3
0.25	4.4	5	-3	18.2	7	-3	-	-	-	-
0.2	-	1	-3	3.8	7	-3	-6	-3	-3	-3
0.1	-	-3	-3	-	-1	-3	-	-	-4	-3
0.05	-	-4	-3	-	-4	-3	-	-	-	-
0 (control)	4.7	-6	-3	4.7	-6	-3	-6	-3	-6	-3

reason, the DSC analysis for T_{CO} was not very useful for 20% esters blends with less than 1% MG. However, distinctive MG peaks were not observed with the samples with less than 0.25% monopalmitin and with less than 0.20% monostearin. Apparently, the cloud of crystals that was observed in measuring the CP of the samples has a very low melting enthalpy so that the melting of the crystals were not detected as a distinctive peak in the DSC thermograms For this reason, the trends of the CP and T_{CO} data did not agree well.

3.4.2 Monoglyceride and Diglycerides Formed During Incomplete Transesterification

Diglycerides are generally observed to have lower crystallization temperatures than MGs. For example, the β phase of monopalmitin crystallizes at 77°C and the β phase of dipalmitin crystallizes at 63.5°C [6]. It was surprising to note that monopalmitin and dipalmitin had similar effects on the CP of biodiesel as indicated in Table 11. One explanation for this is that the saturated DGs are produced in much greater amounts than MGs. To determine the amounts of MG and DG produced by the transesterification reactions and whether they were of similar fatty acid composition to the soybean oil, a series of tests were conducted to determine the composition of the MGs and DGs formed during incomplete transesterification.

Different completion levels of transesterification were achieved by conducting the esterification reaction at different soybean oil/methanol ratios (90%, 100%, 110%, 130%, 150%, and 200% of the theoretical methanol amount). After the reaction was complete, the methyl esters were washed with distilled water and vacuum dried. The MGs and DGs in the esters were isolated on 1-mm silica gel TLC plates, developed with hexene:ethyl ether:acetic acid = 50:50:1 solvent, and quantitatively analyzed by gas chromatography with heptadecanoic acid methyl ester as an internal standard.

Table 12 shows the MG and DG contents of esters made at different oil/methanol ratios and their effects on the cloud point and pour point of the esters. Generally, the MG and DG decreased with increasing methanol/oil ratio as would be expected from a more complete reaction. Diglyceride was found in higher concentration than MG in esters made from all incomplete reactions, but the DG/MG ratio decreased with increasing completion of the reaction. At 200% theoretical methanol, no MGs and DGs were detected. The pour points of the esters were not affected at any levels of MG and DG contamination tested. The MG and DG mixture with up to 0 8% MG and 1.69% DG had no effect on the cloud point of the esters. Although MGs and DGs were detectable in esters made at 130% and 150% theoretical methanol amount, their presence did not contribute to the crystallization of the esters. The effect of mixtures of MGs and DGs on the cloud point and pour point of the esters did not seem very great compared with pure MG and DG since only about 16% of the MGs that existed were saturated, and an even smaller percentage of the DGs were saturated (with two saturated fatty acids). Due to the many possible configurations of fatty acids in diglycerides, it was hard to extrapolate the results from one type of pure DG to the mixtures of DGs unless all possible combinations have been studied individually. Therefore, studying the effect of diglycerides on the crystallization properties of esters is more difficult than that of monoglycerides.

Table 12. Monoglyceride and diglyceride contents of esters made at different oil/methanol ratio and their effects on the cloud point (CP) and pour point (PP) of esters.

% Theoretical MeOH amount		Fatty Acid Profile					% in esters	DGs / MGs	C P (°C)	P P (°C)
		16 0	18.0	18.1	18:2	18:3				
90%	MG	10.7	4.8	23.0	54.0	7.6	2.60	3.8	7	-3
	DG	9 9	4.2	23.4	54.9	7.6	9.85			
100%	MG	10.6	4.9	23.7	54.1	6.7	1.83	3.0	3	-3
	DG	10.2	4.3	23.8	54.8	6.9	5.57			
110%	MG	10.6	4 8	23.5	53.9	7.2	1.53	2.6	2	-3
	DG	10 2	4.4	23.5	54.9	6.9	3.93			
130%	MG	11.7	7.0	22.7	50.5	8.1	0.73	2.1	-1	-3
	DG	10.4	5.3	23.3	53.1	8.0	1.51			
150%	MG	11.2	6 2	23.3	52.0	6 1	0 80	2.1	-1	-3
	DG	10.5	5 0	23.2	54.0	7.3	1.69			
200%	MG						0 00	-	-1	-3
	DG						0 00			

3.4.3 The Effect of DG/MG Ratio on Crystallization

To determine the effect of mixtures of MG and DG that were isolated from soy esters on the crystallization onset temperature (Tco) of neat esters, 50 g of methyl esters made at 100% of the theoretical amount of methanol were added to a column packed with 100 g of alumina. One liter of solvent (ethyl ether:hexane=10:90) was used as the mobile phase to elute the methyl esters, followed by 1 liter of ethyl ether:hexane=50:50 to elute the DGs (contaminated with methyl ester). Finally, 500 mL of methanol was used to elute the MGs (contaminated with DGs). The collected fractions containing MGs and DGs were evaporated, dissolved in a small amount of chloroform, and applied on 1-mm silica gel TLC plates to further purify the MGs and DGs. MGs and DGs isolated from TLC plates were added to neat methyl esters (from Interchem) at DG/MG ratios of 2 and 3 and a total DG amount of 1.5%, 2.5%, 3.5%, 4.5%, 5.5%, 6.5%, and 7.5%. A Perkin-Elmer Series 7 DSC was used to measure the crystallization onset temperature of the

methyl esters. The samples were heated from -70°C to 90°C at a heating rate of 5°C/min. The Tco was determined as the end point of the highest melting transition, that is, the lowest temperature at which the sample was completely liquid.

The effect of MG and DG mixtures isolated from soy esters on the crystallization onset temperature (Tco) of neat esters is presented in Table 13. At a DG/MG ratio of 3.0, a MG concentration up to 1 5% and DG concentration up to 4.5% had no effect on the Tco of the esters, and the Tco was even slightly lower than that of the control. This may have been caused by the melting point depression effect of the impurities. At higher MGs and DGs concentration, a very small and flat peak indicating a higher melting transition than the methyl esters was observed, but because the samples were mixtures of MGs and DGs, the peaks were even broader and harder to distinguish from the baseline than the pure MG species, as discussed above. At a DG/MG ratio of 2.0, the Tco was significantly increased at all levels of MG and DG compared with the control, but no differences were found among the different levels. The results suggest the possibility that the presence of DGs interferes with the crystallization of MGs. Diglycerides have been reported to inhibit or retard the phase transition of triglycerides [7,8]. Further studies are needed to confirm the interfering effect between DGs and MGs.

Table 13. The effect of MG and DG mixtures isolated from soy methyl esters on the crystallization onset temperature (Tco) of neat esters.

DG/MG ratio, % DG	Actual % MG	Actual % DG	Actual DG/MG ratio	Tco (°C)
3.0, 1.5%	0 5	1.5	3.0	5.2
3 0, 2.5%	1.0	3.3	3.3	4.7
3 0, 3.5%	1.1	3 5	3.2	4.4
3 0, 4.5%	1.5	4.5	3.0	4.1
3.0, 5.5%	1.8	5.5	3.1	16.3
3.0, 6.5%	2 0	6.5	3.3	29.2
3.0, 7 5%	2.5	7.5	3.0	29.2
2.0, 1.5%	0.7	1 5	2.1	28.7
2 0, 2.5%	1.3	2.5	1.9	29.2
2.0, 3.5%	1.8	3.5	1.9	28.7
2.0, 4 5%	2.2	4.5	2.0	29.2
2.0, 5.5%	2.6	5.5	2.1	29.2
2 0, 6 5%	3.2	6 5	2.0	28.9
2.0, 7.5%	3.6	7.5	2.1	28.8
Control (neat, methyl esters)				6.6

3.4.4 The effect of bound glycerin on the viscosity of biodiesel and diesel fuel

Tests were conducted with 0%, 0.1%, 0.5%, 1%, 2%, 3% and 5% monoglycerides (by weight) added to #1 diesel fuel, #2 diesel fuel, 20% biodiesel in #1 diesel fuel, 20% biodiesel in #2 diesel fuel and 100% biodiesel. A total of 35 samples were prepared. Each sample was heated to about 70 ^{0}C in order to completely dissolve the MGs into the fuel. At room temperature a higher percent of MG in the fuel makes a very thick gelatinous mixture, but a lower percent of MG forms crystals in the bottom of the sample. The monoglycerides were derived from soybean oil but had been hydrogenated. Their composition was: 10 8% monopalmitin, 12.0% monostearin, 76.0% isomers of monoolein, and 1.1% isomers of monolinolein.

After the samples were prepared, each sample was heated to between 40°C and 45°C before being loaded to the viscometer. The loaded viscometer was then placed in a constant temperature bath to equalize the sample temperature with the bath temperature. The temperature of the bath was maintained at 40 (± 0 1) °C The viscosities of the samples are shown in Table 14.

At 40°C, 2%, 3% and 5% MGs and #1 diesel fuel, the mixture forms crystals that separate from the fuel and plug the viscometer. A similar effect was found for 3% and 5% MG in No. 2 diesel fuel and 5% MG in a 20% biodiesel blend with No. 2 diesel fuel. At 40°C, 0% to 5% MGs in 100% biodiesel did not form any crystals The viscosity increased as the percentage of MG was increased for all the fuels tested. However, the increases were not large and, if crystals are not present, should not affect engine operation.

3.4.5 Discussion of the Bound Glycerin Specification

If all of the 0.24% bound glycerin allowed by the NBB specification were present as MG, then this would correspond to 0 92% MG. However, in soybean oil-based biodiesel, only about 15% of the MGs are saturated. The data presented in Table 11 indicated that saturated MG amounts over 0.25% for monopalmitin and 0.2% for monostearin would begin to crystallize

Table 14. Effect of adding monoglycerides on the viscosity of diesel fuels and methyl esters. Viscosities in cS. All viscosities measured at 40°C.

	Fuels blended with monoglycerides				
% MG	No. 1 Diesel	No. 2 Diesel	Methyl Soyate	20% MS/80% No. 1 Diesel	20% MS/80% No. 2 Diesel
0 0	1.565	2.546	4.147	1.856	2.756
0 1	1.600	2 576	4.282	1.841	2.800
0 5	1.603	2.578	4.343	1.875	2.849
1 0	1 713	2.624	4.513	1.872	2.854
2 0	Crystals	2 881	4 406	1.931	2.993
3.0	Crystals	Crystals	4.405	2.013	3.009
5 0	Crystals	Crystals	4.607	2.141	Crystals

according to the crystallization onset temperature. The cloud point indicates that crystals will form even at saturated MG concentrations as low as 0.05%. If all of the bound glycerin is present as MG, and the MG composition is the same as for soybean oil, the amount of monopalmitin and monostearin should be about 0.10% and 0.04%, respectively. This should raise the cloud point of the fuel by 2-3°C. This would probably not create any problems but any increase in MG beyond this level is not acceptable.

When mixtures of MGs and DGs were investigated, Table 12 showed that the bound glycerin produced from a transesterification reaction with 130% of the theoretical amount of methanol gave 0 73% MG and 1.51% DG. This translates into 0.42% total bound glycerin and no change in the cloud point. This suggests that the MG and DG interact in a way that inhibits crystallization, and that the specification for bound glycerin could be raised However, when this interaction was investigated with the data presented in Table 13, it was found that the crystallization onset temperature depends on the ratio of DG to MG. When 0.7% MG and 1.5% DG were tested, the T_{CO} was 28.7°C compared with 4.7°C for the baseline ester.

The interaction between MGs and DGs that determines their crystallization properties is complex and a complete study was beyond the scope of this project. However, the data presented here indicate that the level of bound glycerin is close to the limit at which crystallization will occur and the apparent discrepancies between the CP and T_{CO} would need to be resolved before the specification value should be raised.

3.5 Alcohol

Methyl alcohol is the most common alcohol used for producing biodiesel although ethanol is used in some cases. Our measurements indicate that when the tranesterification of soybean oil is conducted with 100% excess methanol, about half of the excess methanol is in solution with the ester phase and half is with the glycerin phase. If the biodiesel is washed with water when the tranesterification reaction is complete, there should be very little alcohol left since the alcohol is more soluble in the water than in the biodiesel. Further, if the biodiesel is vacuum dried to remove the residual water from the washing operation, any remaining methanol should be removed

The only fuel or engine parameter likely to be affected by the residual alcohol is the flash point. High concentrations (>5%) of methanol could have an impact on fuel lubricity and cetane number but low concentrations (<1%) should have no measurable effect, Figure 3 shows the flash point of biodiesel with varying amounts of methanol. If 100°C is set as the minimum allowable flashpoint for biodiesel, this will be exceeded by a methanol fraction between 0.18 and 0.22%. The allowable limit for ethanol is slightly higher at 0.26%. Since there is no known deleterious effect associated with these small concentrations of methanol, there does not seem to be any reason to place any restrictions on alcohol except as dictated by the flashpoint restriction. Fuel suppliers needing to satisfy a 100°C flashpoint specification, the current limit in the National Biodiesel Board specification will need to get the methanol concentration below about 0.2% and this should ensure there are no lubricity or cetane number problems associated with high concentrations of alcohol.
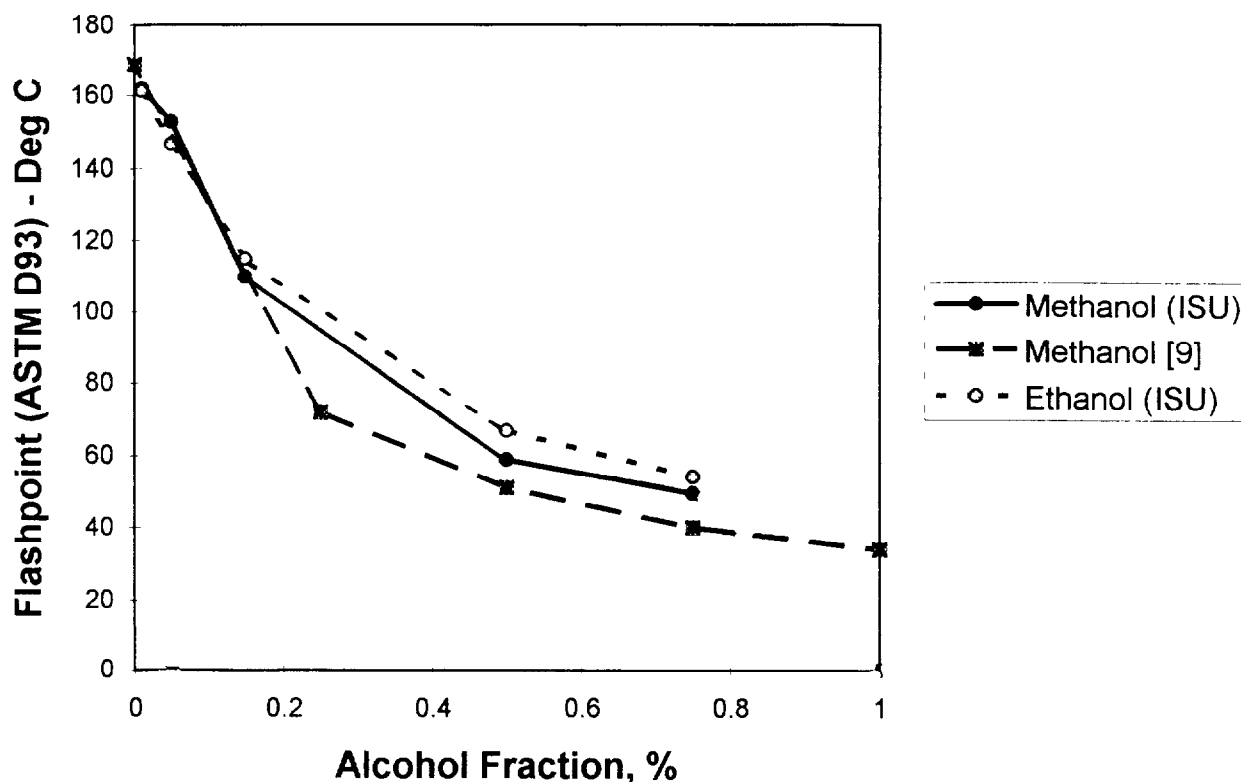
Figure 3   The effect of alcohol on the flashpoint of biodiesel

3.6 Free fatty acids, soap, and catalyst

Free fatty acids, soap, and catalyst are treated together because they are related compounds. Most biodiesel is produced using an alkaline catalyst such as sodium methoxide, sodium hydroxide, or potassium hydroxide. Any free fatty acids that are present will react with the alkaline catalyst to form soaps. These soaps are then removed during the washing process. The washing process also removes any residual catalyst.

The primary problem associated with these contaminants is that when the fuel burns in the engine, the metal remaining from the soap or catalyst will be converted to ash. The current specification for the ash content of biodiesel is 0.02% although this is believed to be an error. It should be no greater than the 0.01% given by ASTM D 975, the standard for No. 2 diesel fuel.

To investigate the amount of residual catalyst and soap formed during the transesterification reaction, an experiment was conducted to determine the effectiveness of water washing. A batch of methyl soyate was produced from a food-grade soybean oil using potassium hydroxide as the catalyst. The methyl soyate was separated from the mixture of methyl soyate, soap and catalyst by washing with distilled water. The steps of the washing process were as follows:

1   Add 100 cc of distilled water to a 500 ml separatory funnel containing approximately 200 cc of raw methyl soyate (mixture of methyl soyate, soap, catalyst, and unreacted methanol) Shake the distilled water and the raw methyl soyate mixture.

2   Wait until the soapy water separates from the mixture.

3   Separate the soapy wash water from the mixture and place into a 150 ml flask for later soap and catalyst tests. The soapy water also contains a portion of the unreacted methanol and catalyst.

4   Repeat steps 1-3 seven times and keep all the wash water samples in 150 ml flasks for measurement of soap and catalyst.

The amount of soap and residual catalyst in the methyl soyate was determined by adding a sample of the wash water to a larger quantity of ethanol and titrating with 0.01N HCl using a phenolphthalein indicator solution. The amount of residual catalyst was calculated from the formula:

$$\% \, Catalyst \, (as \, potassium \, hydroxide) = \frac{Q_1 \bullet 56.1}{1000q} \bullet 100$$

where Q_1 = milliequivalents of 0 01N HCl added (milliequivalent = ml of HCL * Normality of HCL)

q = sample size in grams

For the soap measurement, 4-5 drops of bromophenol blue indicator solution was added and the titration continued to the yellow end point. The amount of soap was calculated from the formula:

$$\% \, Soap \, (as \, sodium \, oleate) = \frac{Q_2 \bullet 304.4}{1000q} \bullet 100$$

where Q_2 = milliequivalents of additional 0 01N HCl added.

The percentage of soap and catalyst in the unwashed methyl soyate, wash water, and glycerin are shown in Table 15. The unwashed methyl soyate was found to have 0.149% soap content and no measurable catalyst. When this methyl soyate was washed, the first batch of wash water (100 cc of distilled water) contains most of the soap and catalyst. The second and the third wash water contain less soap and no measurable catalyst. The other batches of wash water contain essentially no soap or catalyst. The level of soap indicated is equal to that measured on the distilled water used for washing and is probably due to a bias caused by the phenolphthalein solution. For the calculations described later, the soap shown for distilled water was subtracted from the other quantities. Table 15 also shows that most of the soap and catalyst are present in the glycerin phase.

The methanol present in the glycerin phase can be separated by heating the mixture in a vacuum. The yield of methyl soyate, glycerin, soap, and a potassium balance are shown in Table

Table 15. Percentage of soap and catalyst in wash water, unwashed methyl soyate and glycerin.

Sample	% Soap	%Catalyst
Unwashed methyl soyate	0.149	0
1st washing	0.249	0.015
2nd washing	0.038	0
3rd washing	0.018	0
4th washing	0.015	0
5th washing	0.015	0
6th washing	0 015	0
7th washing	0 015	0
distilled water	0.015	0
glycerin phase	6.058	7.173

Table 16. Total mass of products and potassium balance

Total amount of methyl soyate	170.74 g
Glycerin and unreacted methanol mixture	30.25 g
Glycerin (after heated in a vacuum)	19 52 g
Unreacted methanol in glycerin	10.73 g
Soap in unwashed methyl soyate	0.254 g
Total soap in all 7 samples of wash water	0.255 g
Total input of potassium (K)	1.24 g
Total output of potassium (K)	1.17 g

Table 17. Composition of glycerin and ester fractions

Glycerin fraction				Ester fraction			
glycerin (g)	catalyst (g)	soap (g)	methanol (g)	methyl soyate (g)	catalyst (g)	soap (g)	methanol (g)
19.52	1.4002	1 183	10.73	170.74	0.015	0.254	did not measure

16. The total amount of the methyl soyate and glycerin produced in the reaction are 170.74 g and 19 52 g respectively. The total amount of soap in the seven batches of wash water is 0.254 g which is virtually the same as the amount of soap measured in the unwashed methyl soyate. This confirms the accuracy of the soap measurements   Also, when the potassium portion of the soap and catalyst are compared to that originally added as catalyst, the agreement is good.

A breakdown of the two phases produced during transesterification is shown in Table 17. This table shows that the ester fraction contains almost no catalyst and about 17% of the total soap produced during the reaction. The glycerin phase contains almost 99% of the residual

catalyst The methanol fraction was not measured during this experiment but later testing has shown that the excess methanol splits fairly evenly between the ester and glycerin phases.

As mentioned earlier, the ash standard for diesel fuel is 0.01%. If this standard is applied to biodiesel, then it is necessary to get the residual catalyst below 0.015% since potassium makes up only about 70% of the catalyst mass. The measurements presented here indicate that this level is achievable by 1-2 water washes There does not appear to be any reason for a separate specification for soap or catalyst, since the ash standard already keeps these contaminants at a very low level. However, the ash standard given in the National Biodiesel Board specification should be lowered to the ASTM D 975 value of 0.01%.

3.6 Oxidation of Biodiesel

Although not included in the original project description, oxidation was identified during the course of the project as a major source of biodiesel contamination. This section describes the results of initial efforts to determine the significance of biodiesel oxidation on overall fuel quality.

Soybean oil has a higher unsaturation level than many other oils and metal catalysts of oxidation may exist in biodiesel storage tanks, so soydiesel is very susceptible to autooxidation during storage Upon oxidation, the chemical compositions of methyl esters may be changed, and this may in turn alter the properties of biodiesel. When this process occurs at ordinary temperatures, the initial products are hydroperoxides formed by the addition of oxygen to a carbon atom adjacent to a carbon-carbon double bond [10]. Generally, at this step, the double bond is left intact. The extent of this level of oxidation can be characterized by the *peroxide value* As oxidation proceeds, the peroxides may split and form aldehydes and short chain acids.

Oxidation of biodiesel occurs through a series of steps. Most vegetable oils contain natural antioxidants, such as Vitamin E (tocopherol), that inhibit oxidation until the antioxidant is consumed. When the natural antioxidants are depleted, oxidation proceeds rapidly. The period of slow oxidation that precedes rapid oxidation is often called the *induction period*.

Bailey [10] states that if two or more double bonds are present in one fatty acid chain, they have a *mutually activating effect* For example, linoleic acid, with two double bonds, oxidizes more readily than oleic acid with only one double bond and the tendency to oxidize is greater than can be explained by the increase in the number of double bonds.

In the food industry, the result of oxidation is rancidity which is characterized by an unpleasant odor. When esters are used as fuel, an important consequence of oxidation is that the hydroperoxides are very unstable and tend to attack elastomers. In addition, the hydroperoxides can induce polymerization of the esters and form insoluble gums and sediments. Sediment and gum formation can be a problem with diesel fuel also, although the unsaturated nature of soybean-based biodiesel may aggravate the problem.

Recent engine durability testing with soybean-based biodiesel has shown that biodiesel may be subject to fuel filter plugging problems caused by sediment and gum formation [11]. The currently accepted explanation for the presence of sediment and gum is that the fuel changes chemically to produce these compounds and this is identified as a *fuel stability* problem. Biodiesel's oxidation is accelerated by heat and light. Generally, when air is present, oxidation will proceed. The objective of our study was to find the effect of oxidation level on the properties (such as cetane number) of biodiesel

When biodiesel has been distilled to remove the high boiling point materials such as monoglycerides and glycerin, the natural antioxidants such as tocopherol are also removed. In this project, methyl esters freshly prepared from refined vegetable oil (Flavorite brand) were distilled under vacuum to remove pigments, polymers, and natural antioxidants. The distillates were collected from 120°C-150°C at less than 0.05 torr. Peroxide values (PV) and fatty acid composition of the esters were measured just before and after distillation and are shown in Table 18. The fatty acid composition of the esters before and after distillation were similar. After distillation, the PV of the esters was zero. Heating of the esters in the absence of air or oxygen might have caused the decomposition of the peroxides.

The freshly prepared and distilled methyl esters were oxidized under fluorescent light with continuous stirring at room temperature. The peroxide value was measured every two days by the AOCS Official Method. The oxidation curve for distilled and nondistilled methyl esters is presented in Figure 4. The peroxide value of the freshly prepared, nondistilled esters increased almost linearly with increasing time during the oxidation period. It took 24 days for these esters to reach a peroxide value of 80. However, the oxidation of distilled methyl esters was much faster. It took only 6 days to reach a peroxide value of 96. Distillation apparently removes the naturally occurring antioxidants without changing the fatty acid composition of the esters.

It was suspected that changing the oxidation level of the biodiesel would have an impact on the cetane number since peroxide compounds have been proposed as cetane improvers [12]. Table 19 shows the results of cetane number measurements on the samples described in Figure 4, before and after oxidation.

As can be seen in the table, when the freshly prepared esters were oxidized to a peroxide value of 81, the cetane number increased from 47.8 to 54.6. A soybean oil with a peroxide value of 81 would generally be considered to be rancid. However, methyl soyate with a high peroxide value does not always have the unpleasant smell usually associated with rancidity.

The cetane testing of the distilled esters showed that when the cetane number was determined before oxidation occurred, the cetane number was the same as before distillation. The decrease from 47 8 to 46.8 is not considered to be significant. However, as with the undistilled esters, there was a large increase in cetane number as the esters were oxidized, from 46.8 to 55.4. While the reason for the cetane number increase is not entirely certain, it is likely that the hydroperoxides formed during the oxidation of the esters are responsible. Dialkylperoxides have been shown to be strong cetane enhancers [12] and hydroperoxides are likely to increase the cetane number also.

To further investigate the effect of oxidation on the cetane number of biodiesel, samples of methyl soyate at six different PV levels were prepared. To produce these samples, freshly

Table 18. The peroxide value and fatty acids compositions of soy methyl esters before and after distillation.

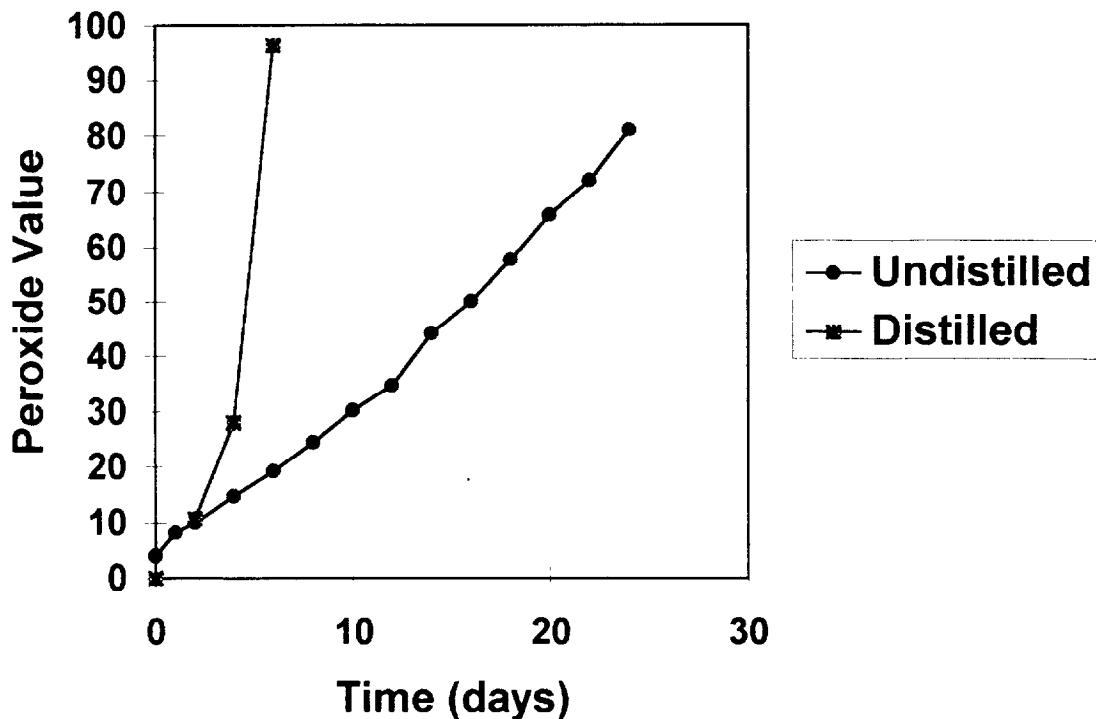| Samples	PV	16.0	18:0	18:1	18:2	18:3
Fresh methyl esters	5.95	10.49%	3 93%	21.66%	55.62%	8.31%
Distilled methyl esters	0	9.57%	4.14%	22.03%	55.86%	8.39%

Figure 4   Rate of increase of peroxide value for distilled and undistilled esters.

prepared, undistilled methyl soyate was mixed in varying amounts with highly oxidized, distilled methyl soyate   Since the previous testing had established that distillation did not affect the cetane number, any changes in cetane number between these samples were assumed to be due to the difference in oxidation. Each of the six samples was split into three parts and the 18 testspecimens were submitted as blind samples to a commercial laboratory for cetane testing. The results of this testing are shown in Figure 5.

Figure 5 shows the three values of cetane number at each of the six levels of peroxide value. ASTM D613 states that since these measurements were conducted by the same operator, on the same equipment, and on the same test material, the difference between the highest and lowest value should be within 0.7 to 0.8 cetane numbers. However, the differences in this data were as large as 3.9. In spite of the experimental variation, the trend toward increasing cetane number with increasing peroxide value is clear.

The equation for a least-squares-fit cubic polynomial is shown in the figure. The predicted

Table 19. Effect of oxidation and distillation on cetane number.

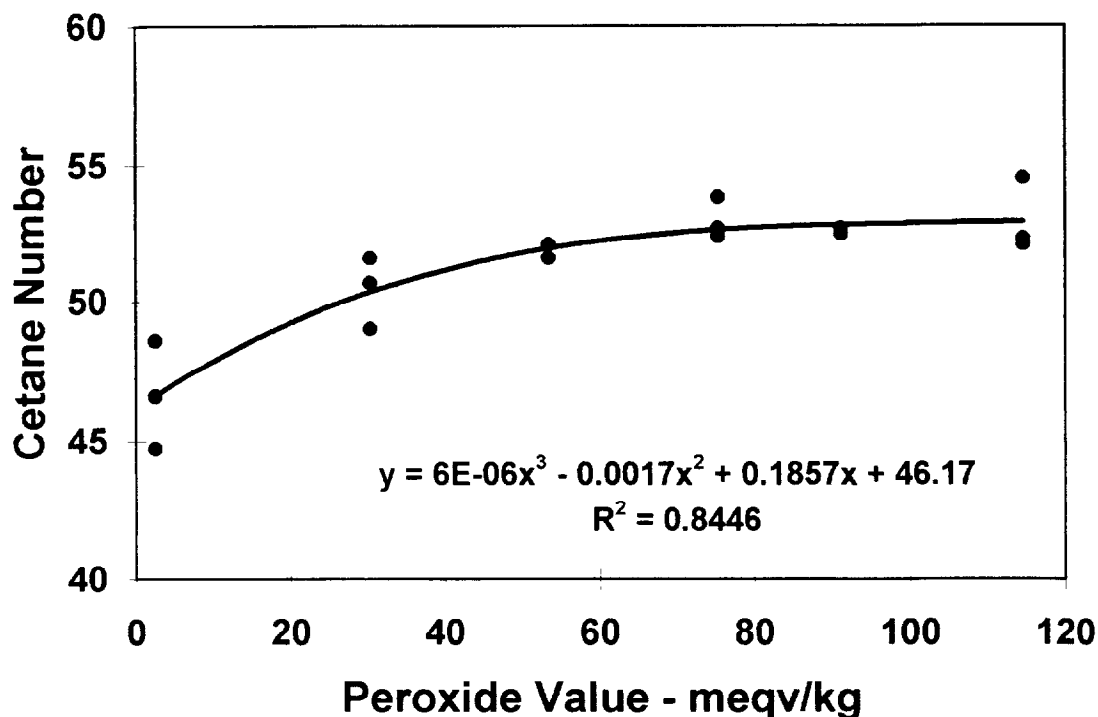| Sample	Rep 1	Rep 2	Average
Freshly prepared methyl esters (PV=6)	46.8	48.7	47.8
Oxidized methyl esters (PV=81)	54 6	54.5	54.6
Distilled methyl esters (PV=0)	46.5	47.2	46.8
Distilled and oxidized methyl esters (PV=82)	55.5	55.2	55.4

Figure 5. The effect of peroxide value on cetane number.

cetane number for methyl soyate with a peroxide value of zero is 46.17. This value corresponds well to the low peroxide value cetane number of 47.8 and 46.8 given in Table 19. It appears that the cetane boosting effect of the hydroperoxides becomes minimal above a peroxide value of 70.

At the present time, the National Biodiesel Board (NBB) specification does not include any standard that relates to oxidation. The European standard on which the NBB specification is based includes a minimum Iodine Value of 115 but this was dropped from the NBB specification because it prevented the use of soybean oil as a feedstock. The Iodine Value of soybean oil is around 130 while the Iodine Value of rapeseed oil, the most common feedstock in Europe, is less than 115. The Iodine Value is related to the degree of unsaturation of the fatty acids and can be related to the tendency of the fuel to oxidize. Generally, fuels with more unsaturation and higher Iodine Values will oxidize more quickly.

A future biodiesel standard should include two additional specifications. It should include a measure of the biodiesel's tendency to oxidize. This could be the Iodine Value, or a variation of an ASTM fuel stability test such as D 2274. The specification should also include a measure of the current level of oxidation, such as the peroxide value, and a limit should be set on the maximum PV of the fuel. While additional testing is needed to identify the maximum acceptable value of PV for the fuel, a PV of 70 may be appropriate since this is the level beyond which the cetane number does not increase. Further increases beyond this level should have only deleterious effects.

4. Conclusions

The following conclusions can be drawn from the work performed in this project:

- No specification value is needed for the amount of unsaponifiable matter in biodiesel as long as the level of unsaponifiable matter is no higher than is present in the original feedstock.
- The current specification for water content in the NBB specification is not compatible with the existing diesel fuel distribution and storage network. Biodiesel that is transported or stored in this system will be in frequent contact with water and will eventually become saturated. When saturated, biodiesel can absorb about three times the amount of water allowed by the current standards for both biodiesel and No. 2 diesel fuel. Either the standard will need to be raised (probably to 1500 ppm) or biodiesel will need to be transported and stored in special, water-free systems that are separate from those used for diesel fuel.
- All biodiesel that will not be used within a short period of its production should be treated with a biocide to prevent the growth of microorganisms. These microorganisms, especially molds, grow more readily on biodiesel than they do on diesel fuel.
- The current specification for free glycerin appears to be adequate and could potentially be relaxed somewhat from 0.02% to 0.05%.
- The amount of alcohol in biodiesel is controlled by the specification on the flashpoint. The current flashpoint limit of 100°C is well above the limits for No. 1 and No. 2 diesel fuels which are 38°C and 52°C, respectively. The flashpoint limit for biodiesel could be relaxed to 52°C, which would allow a higher level of alcohol, without the alcohol rising to level where it is likely to affect the fuel's cetane number or lubricity
- The crystallization properties of pure monoglycerides suggest that the specification value for bound glycerin is at the limit to prevent crystal formation. However, when monoglycerides and diglycerides are considered together in ratios consistent with their presence in methyl esters of soybean oil, the diglycerides appear to inhibit crystal formation by the monoglycerides. Further research into the interactions between the monoglycerides and diglycerides is needed before an increase in the level of bound glycerin should be considered.
- The amounts of free fatty acids, soap, and residual catalyst are not currently included in the National Biodiesel Board specification. This is acceptable as long as an alkaline catalyst is used and the biodiesel is water washed. The specification on ash content will keep these contaminants at a low level. However, if acid catalysts are used, which is likely if waste restaurant grease is used as a feedstock, or if an alternative to water washing is used, then a specification for free fatty acids, soap, and residual catalyst may be necessary.
- The specification for biodiesel should include a limit on the tendency of the fuel to oxidize and a limit on the maximum degree of oxidation allowable for use of the fuel in diesel engines. More research is needed to determine the appropriate tests and limiting values for these properties.