RAPID EVALUATION OF CANOLA LINES FOR COLD SOAK FILTERABILITY IN BIODIESEL

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Hongjian Lin

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Agricultural and Biosystems Engineering

August 2010

Fargo, North Dakota

North Dakota State University
Graduate School

Title

RAPID EVALUATION OF CANOLA LINES

FOR COLD SOAK FILTERABILITY IN BIODIESEL

By

HONGJIAN LIN

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE

ABSTRACT

Lin, Hongjian, M.S., Department of Agricultural and Biosystems Engineering, College of Engineering and Architecture, North Dakota State University, August 2010. Rapid Evaluation of Canola Lines for Cold Soak Filterability in Biodiesel. Major Professor: Dr. Dennis P. Wiesenborn.

Worldwide concerns about fossil fuel depletion and energy security have recently triggered a research interest in biodiesel, which is renewable, biodegradable, and has several other advantages as an alternative to petro diesel. However, biodiesel may cause engine problems, especially fuel filter plugging, associated with its use in cold weather conditions. Trace contaminants such as glycerin, saturated monoglycerides (SMG), and soap compromise cold weather performance of biodiesel. A cold soak filtration test was recently included in the U.S. specifications for biodiesel (ASTM D 6751-09) to evaluate biodiesel cold weather performance.

Canola seed has good potential to be a locally important biodiesel feedstock because of its high yield (1500 to 2200 kg/ha) and oil content (40 to 50%, *Brassica napus* L.), as well as a suitable fatty acid profile for good cold weather performance. For a plant breeding program evaluating canola biodiesel quality traits, rapid preparation of biodiesel samples and assessment of its quality is important. In this work, an *in situ* alkaline transesterification method was adopted for preparing canola biodiesel. It was found that the biodiesel yield via this method was improved by reducing seed moisture from 6.7% to 0% after oven-drying. The resulting biodiesel had qualities comparable to or better than biodiesel prepared through the conventional alkaline transesterification.

Only a limited amount of seed from new canola lines is typically available in a plant breeding program; obtaining the required volume of biodiesel for evaluating cold soak filterability (300 mL) is not possible. In order to rapidly screen canola breeding lines

for B100 quality, cold soak filterability must be assessed with reduced volumes of biodiesel. Therefore, this study evaluated the impact of SMG, glycerin, and soap on cold soak filterability. Biodiesel filtration time rapidly increased to unacceptable levels and became much less reproducible when the SMG concentration was raised above 0.28%. A regression model was generated to predict the filterability of biodiesel against the concentrations of trace contaminants. A downscaled model of the filtration test with a reduced volume of biodiesel sample (25 mL) was also tested and calibrated.

The *in situ* transesterification method saved 30% operator time compared with the conventional method. By combining the downscaled cold soak filtration test, the goal of analyzing 40 biodiesel samples/wk was achieved.

ACKNOWLEDGMENTS

I thank my advisor Dr. Dennis P. Wiesenborn, Professor in the Department of Agricultural and Biosystems Engineering, for his support and guidance through the duration of my two-year graduate study. Working with Dennis was the most valuable experience in my life. I am so fortunate to have Dennis as a model for my future study.

I also thank other advisory committee members: Dr. Scott W. Pryor, Assistant Professor in the Department of Agricultural and Biosystems Engineering; Dr. Burton L. Johnson, Professor in the Department of Plant Sciences; and Dr. Clifford Hall III, Associate Professor in the Department of Cereal and Food Sciences. This thesis would not have been possible without their advice and comments.

Special thanks go to Darrin M. Haagenson, research specialist in the Department of Agricultural and Biosystems Engineering, who initiated the *in situ* biodiesel work and collaborated with me in the experiment. I extend my appreciation to the North Dakota Center of Excellence for Oilseed Development for providing funding.

Thanks to all others who contributed to the completion of this thesis: Rachel Brudvik, research specialist in the Department of Agricultural and Biosystems Engineering; Mukesh Kumar, Katie McKinnon, and Shay Richter, Department of Agricultural and Biosystems Engineering; Leonard Cook, USDA-ARS; Enrico Sassi, English Department; Curt Doetkott, Information Technology Services; Dr. Mukhlesur Rahman, Department of Plant Sciences; and Jana Daeuber, Department of Agricultural and Biosystems Engineering. It was a great pleasure to work with all of these professionals.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Concerns about fossil fuel depletion and energy security, and the demand for rural economic development have triggered a blooming research interest in biodiesel in recent years. Biodiesel is a form of fatty acid methyl esters (FAME). It has various advantages as a renewable fuel for transportation use: 1) derivation from renewable domestic resources; 2) biodegradability; 3) reduction of most exhaust emissions; 4) higher flash point which leads to safe handling; and 5) excellent lubricity (Knothe, 2005).

The biodiesel production capacity and estimated production (Figure 1) in recent years in the US have rapidly increased (Brown, 2009). However, the industry experienced a low utilization rate of the production capacity in 2009 and 2010 due to feedstock shortage (partly due to higher price for other uses, e.g., for cooking oil), current economic conditions, and less favorable public policies. Efforts on exploiting different and sufficient agricultural or industrial raw materials as biodiesel feedstocks have been made. In North Dakota, canola seed (*Brassica napus* L.) is a promising biodiesel feedstock. In 2007, the Archer Daniels Midland (ADM) company established a biodiesel plant based on canola seed with a capacity of 85 million gallons per year in Velva, ND (ADM Company, 2007). Despite various advantages, cold weather performance of biodiesel is increasingly perceived as a big concern in practice when it is used in winter conditions in the Northern Plains of the US. In the winter of 2008, at least 15 cases of vehicle fuel filter plugging problems were linked to biodiesel blends in Minnesota (MDA, 2009). Therefore, when developing suitable biodiesel feedstocks, adequate cold weather performance is desirable.

Canola is suitable as a biodiesel crop due to its high yield (1.5 to 2.2 ton seed/ha) and oil content (40 to 50%), and characteristic fatty acid (FA) profile (USDA, 2009).

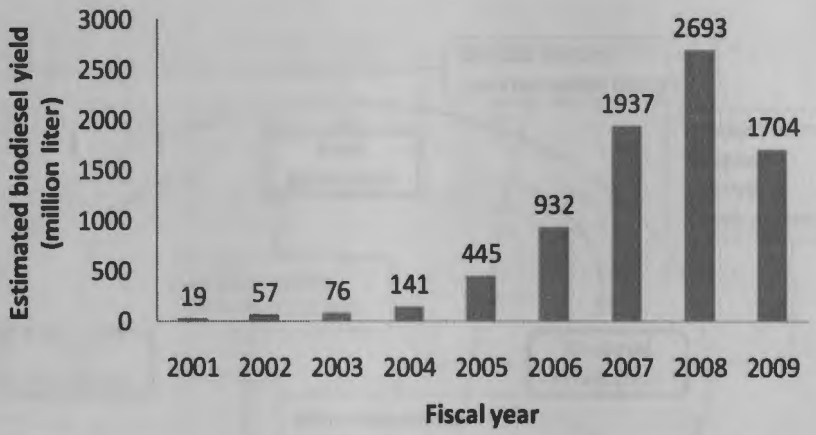

Figure 1. Estimated biodiesel production in recent years in the US by fiscal year (Brown, 2009).

Soybean is currently the major biodiesel feedstock in the US. However, the oil content of canola greatly exceeds that of soybean (20%). Furthermore, the lower content of saturated fatty acid and higher content of monounsaturated fatty acid (oleic acid, C18:1; 60%) in canola oil results in a better cold weather performance and oxidative stability of its biodiesel.

However, further screening and assessment practices are needed when canola breeders want to determine which canola lines result in better biodiesel qualities. Researchers often focus on the FA profile of the parent oil, which influences some biodiesel quality traits, such as kinematic viscosity, cloud point, and oxidative stability. But the FA profile is just one of many factors which influence the fuel quality. The combined effect of the canola genetic and environmental factors may have a complex influence on biodiesel properties. Therefore, a screening process to determine the relationship between the influential factors and biodiesel properties will be welcome and valuable to the canola breeders, farmers and biodiesel producers (Figure 2).

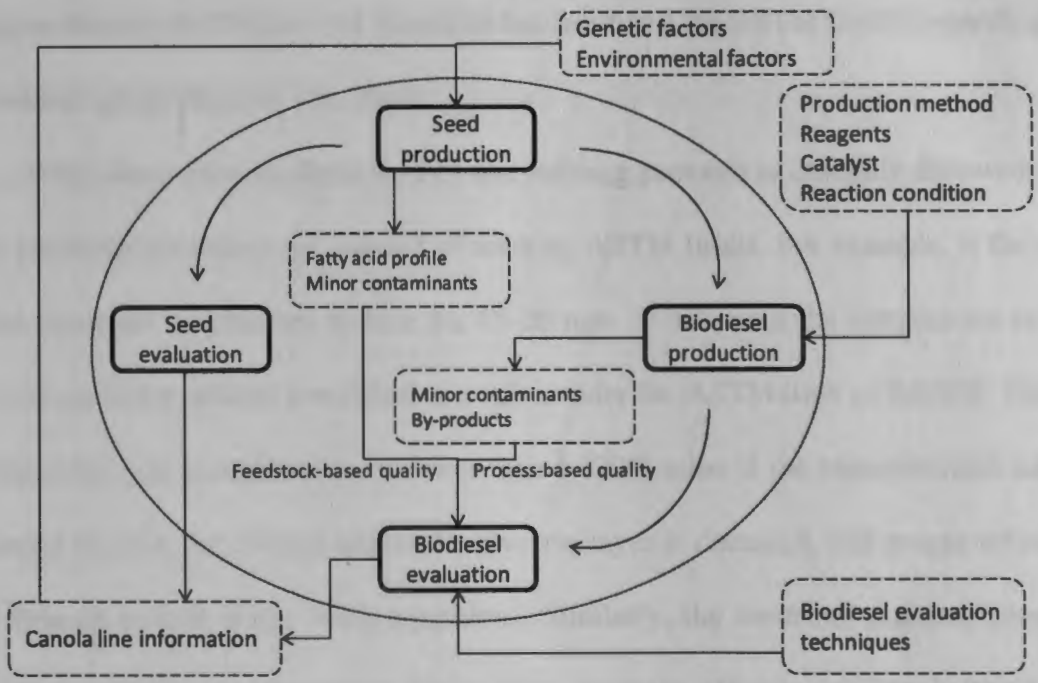

Figure 2. Flowchart for screening canola seed for biodiesel production.

At the outset of this study, the assessment of biodiesel properties (e.g., oxidative stability, kinematic viscosity, and free and total glycerin) had already been partly realized at North Dakota State University, but the biodiesel preparation methods were very time consuming. In contrast, yield of canola seed, the oil content, and the FA profile, which are fundamentally important quality indicators for their suitability for biodiesel production, were being obtained via a high throughput technique (600 samples per wk). A baseline screening procedure for biodiesel quality is summarized as follows. Canola seeds are moisture-conditioned and screw-pressed. The resulting oil is then alkaline transesterified with methanol into FAME. After glycerin and excessive methanol are removed, the crude biodiesel is subjected to a water wash to remove the remaining glycerin, catalysts and methanol, and finally dried by heating, and stored in containers kept from light and heat

(20°C). The produced biodiesel is then analyzed to obtain property information. The American Society for Testing and Materials has instituted the ASTM D 6751 specifications for biodiesel properties (ASTM, 2009).

When the transesterification (TE) and refining protocol is carefully followed, most of the biodiesel properties are assured of meeting ASTM limits. For example, if the water washed biodiesel is subjected to heat for 15-20 min 17 kPa until the temperature reaches 95°C, the moisture content is reduced to a value under the ASTM limit of 0.050%. The free glycerin content is guaranteed to be lower than 0.020% mass if the transesterified mixture is allowed to settle for 30 min before the glycerin layer is decanted, and proper refining is done through several water wash repetitions. Similarly, the methanol content, kinematic viscosity (at 40°C), cetane number, acid number, and total glycerin are found always to be within the ASTM D 6751 limits if a good TE and refining process is applied. Therefore, these biodiesel quality traits tend to be more process-based, which means they should be within the ASTM limits when a sound, established protocol is applied. Cold soak filterability and oxidative stability of biodiesel are very important quality traits. They are partly feedstock-based, and that means they may significantly vary due to the variability in feedstock oil. Moreover, preliminary data about these properties have not been published extensively. These properties should be evaluated to ensure that advanced canola lines (*Brassica napus* L.) are suitable for biodiesel use.

Challenges are encountered when hundreds of canola lines must be evaluated for their suitability for biodiesel use, because the time required by the screening process described above creates a bottleneck: the conventional TE and refining process is time-consuming and lacks flexibility; some of the biodiesel quality tests require large amounts

of biodiesel samples, which are impossible to obtain from canola breeders. Due to the importance of cold weather performance of biodiesel, the main focus of this study is on the test of cold weather performance. To resolve the problems mentioned above, an *in situ* TE method (Figure 3) and downscaled cold soak filtration test are proposed in this study.

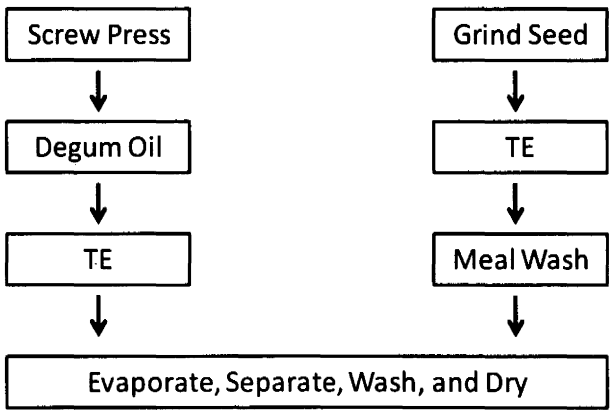

Figure 3. Scheme for a baseline conventional alkaline TE (left) and an *in situ* TE (right) for biodiesel preparation.

Objectives of the Study

The objectives of this study were to develop a screening protocol for characterizing properties of canola biodiesel, and to realize biodiesel preparation from a small amount of canola seeds in an efficient way. Biodiesel yield of *in situ* TE method was evaluated for canola seeds with different moisture levels (from 0 to 7%), and both time requirement and biodiesel properties of the *in situ* and conventional TE methods were compared. The cold soak filterability test adequately measures cold weather performance of biodiesel, but each measurement requires 300 mL of sample which is often not available from experimental canola lines. In order to prepare model biodiesel for scaling down the cold soak filtration test (ASTM D 7501-09b), trace contaminants (saturated monoglycerides, glycerin, and

soap) were evaluated for their effects on biodiesel cold soak filterability. The goal was to develop techniques that would allow for the evaluation of 40 seed samples per week at a seed testing lab at NDSU.

Thesis Organization

The thesis is divided into a literature review and two research papers. The literature review discusses the biodiesel preparation and characterization methods that might be used to develop a higher throughput screening tool for canola seed. The *in situ* transesterification showed good potential to be incorporated in canola breeding programs. The cold weather performance was identified as challenging biodiesel quality trait, and found to be worthy of an in-depth study. Paper 1, entitled "Yield and Characteristics of Canola Biodiesel Prepared through Conventional and *in situ* Transesterification with Various Seed Moisture Contents," details the establishment of *in situ* alkaline TE procedure for canola biodiesel preparation, and compares important quality traits of biodiesel prepared through the *in situ* and conventional TE methods. Paper 2, entitled "Effect of Trace Contaminants on Cold Soak Filterability of Canola Biodiesel," details the impacts of three trace contaminants on biodiesel cold soak filterability, and presents a procedure to evaluate filterability with a downscaled model for cold soak filtration test. The future research areas are suggested after the two papers. Appendices show experiment designs, original data, and data analysis results.

References

ADM Company. 2007. ADM opens biodiesel production facility in Velva, North Dakota. Available at: http://www.adm.com. Accessed 20 Sept 2008.

ASTM. 2009. Standard specification for biodiesel fuel blend stock (B100) for distillate fuels, designation D 6751-09. West Conshohocken, Pa.: American Society for Testing and Materials.

Brown, L.R. 2009. *Plan B 4.0: Mobilizing to Save Civilization*. New York, N.Y.: W.W. Norton & Company.

Knothe, G. 2005. Introduction. In *The Biodiesel Handbook*, 1-3. G. Knothe, J. Krahl, and J. Van Gerpen, eds. 1st Ed. Champaign, Ill: AOCS Press.

MDA. 2009. Report to the legislature: petroleum diesel fuel and biodiesel technical cold weather issues. St. Paul, Minn.: Minnesota Department of Agriculture.

USDA. 2009. North Dakota Canola Production Statistics: 2008. National Agricultural Statistics Database. Washington, D.C.: USDA National Agriculture Statistics Service. Available at: http://www.nass.usda.gov. Accessed 5 Jan 2010.

LITERATURE REVIEW

Canola, a cultivar of rapeseed, is an important oilseed crop with improved quality traits compared to rapeseed. The high level of erucic acid in rapeseed oil was perceived to be linked to heart disease in consumers. Rapeseed crop breeding programs therefore produced low erucic acid varieties in the 1960s in Canada. The rapeseed meal after oil extraction was used as animal feedstuff, but it had a high level of glucosinolates, which potentially caused problems of palatability and was associated with goitrogenic, liver, and kidney abnormalities of livestock. Further breeding development then produced the double-low varieties, both low in erucic acid and low in glucosinolates, in Canada in 1974, which were named "canola" (Booth and Gunstone, 2004). The most common species of canola are *Brassica napus* L. and *Brassica rapa* L., and both of them can be categorized into several subspecies such as winter and summer types. Almost all current production, 99% in 2009, of rapeseed in the United States is canola. North Dakota canola production (*Brassica napus* L.) accounted for 88.5% of the US national acreage with 2.89×10^3 ha harvested in 2009 (USDA, 2009).

With the increasing demand for canola oil as a food and fuel source, there are interests in breeding and expanding canola production. Canola breeding efforts continue to identify elite lines that will perform well across environments possessing potential variability in temperature and available precipitation. Winter canola has been evaluated in North Dakota trials, and winter canola is included in crop rotations in several Southern Plains states (Duke et al., 2009). The influence of production environment or genotype on fatty acid (FA) composition or plant metabolism has been well documented in many crop

species, but the impact of canola production environment and variety selection on biodiesel quality has not been extensively investigated.

There are several ways to modify vegetable oil to make it suitable for diesel use. Biodiesel, which in this article refers to fatty acid methyl esters (FAME), is a category of chemicals derived from triglycerides (TG) by transesterification (TE). The TE process is the most intensively explored way to produce biodiesel, and the TE-produced biodiesel generally performs well when it meets with the ASTM D 6751 B100 specifications (ASTM, 2009).

The focus of this study was to develop methods to screen new canola lines for biodiesel production. Canola yield per ha, seed oil content, and FA profile of canola oils are important parameters for determining a canola line's suitability for biodiesel use. These agronomic parameters are critical for the commercial success of a new canola line, but they are not the only consideration, because the ASTM D 6751 requires that biodiesel meet strict quality standards. Therefore, important biodiesel quality traits (cold weather performance, oxidative stability, kinematic viscosity, free and total glycerin, and etc.) should be tested in addition to the agronomic parameters. Furthermore, both the biodiesel production method and minor impurities in biodiesel have impacts on its fuel qualities. Properties of canola oil, common biodiesel production methods and characterization methods are reviewed and discussed below.

Canola Seed as Biodiesel Feedstock

Oil composition and use

Canola oil was originally developed for food use, but it is also considered to be a good feedstock for industrial products, such as biodiesel, lubricants, surfactants, paints and

inks, and polymers (Walker, 2004). Recent consumers' and scientists' interest in transportation fuels stimulated studies on canola oil for biodiesel use. Compared with other crops, canola oil is low in palmitic and stearic acid, which are saturated, and high in oleic acid, which is monounsaturated (Table 1). The high oleic acid makes canola an ideal biodiesel feedstock due to potential excellent cold weather performance. Besides the above-mentioned major fatty acids, canola oil contains a number of minor fatty acids (mainly *n*-7 series of monoethylenic fatty acids) which account for 2-3% of total fatty acids (Ratnayake and Daun, 2004).

Table 1. Typical fatty acid profiles (wt. % of total fatty acids) of soybean oil, sunflower oil, cottonseed oil, canola oil and tallow (O'Brien, 2009).

Characteristics [a]	Soybean oil	Sunflower oil	Cottonseed oil	Canola oil	Tallow [b]
Palmitic	10.6	7.0	21.6	4.1	24.3
Stearic	4.0	4.5	2.6	1.8	21.4
Oleic	23.3	18.7	18.6	60.9	33.6
Linoleic	53.7	67.5	54.4	21.0	1.6
Linolenic	7.6	0.8	0.7	8.8	0.6
Others	0.8	1.5	2.1	3.4	18.5

[a] Palmitic (C16:0); Stearic (C18:0); Oleic(C-18:1);Linoleic (C18:2);Linolenic (C18:3).
[b] The tallow contains significant amount of C-18:1, C-18:2, and C-18:3 *trans* fat.

Some non-lipid trace components in canola oil may play a role in its resulting biodiesel characteristics. Chlorophyll pigment, a pro-oxidant, as well as breakdown products of chlorophyll pigment, are suspected to cause oxidation problems with the oil and esters (Kulkarni et al., 2006). Chlorophyll pigment content in canola oil is dependent on the amount of green seeds in the harvested seeds, which results from inappropriate harvest times (i.e. too early harvests) and possibly from alternative harvest methods (straight-cutting and swathing) (Tostenson et al., 2007). But canola oil also contains natural antioxidants such as tocopherols which help prevent the propagation of oil oxidation and

improve its oxidative stability; tocopherols occur as a family of four analogues with a concentration ranging from 430 to 2680 ppm in crude (unrefined) oil.

Waxes, present mainly as sediments on the bottom of oil containers, are wax esters made up of fatty acids and fatty alcohols ranging from C16 to C30 (Ratnayake and Daun, 2004). Wax content may be associated with drought and high temperature conditions where canola crops grow (Botha et al., 2000). There is no research reporting the impact of wax on biodiesel cold weather performance, but it is possible that waxes crystallize with saturated fatty acids to impair biodiesel filterability when temperature decreases to about the cloud point. Policosanol, a set of higher aliphatic primary alcohols which recently showed significant therapeutic efficacy (Viola et al., 2008), could hopefully be derived and refined from canola oil wax esters as a high value-added product.

Oil processing

The main products derived from canola seed are canola oil and meal. Canola oil can be processed to food or industrial products, and canola meal is a good animal feedstuff with high protein content. Oil extraction and refining is therefore required to fractionate oil and meal from canola seed. This processing begins with seed pretreatment, and includes the following steps: seed cleaning, tempering, dehulling, flaking, and conditioning (Figure 4). The seed cleaning is to remove weed, dust, soil, and other contaminants by aspiration and screen separation. Seed is generally cleaned to < 2.5% dockage. The tempering uniformly heats seed to 30 to 40°C for 30 to 45 min, which helps avoid shattering during flaking. The dehulling is achieved by mechanical separation and air aspiration to reduce hull impurities in seed, therefore improving canola meal quality and reducing subsequent processing cost (Ikebudu et al., 2000). The purpose of flaking is to disrupt canola seed structure and disrupt

oil bodies to render a better oil extraction. Flaking is accomplished by passing seeds through iron rollers, achieving a flake thickness of 0.30 mm. The thermal conditioning is then used to further disrupt the oil bodies, to adjust moisture content, and to deactivate enzymes within seeds. It is achieved by heating seed flakes to 75 to 85°C (Booth, 2004).

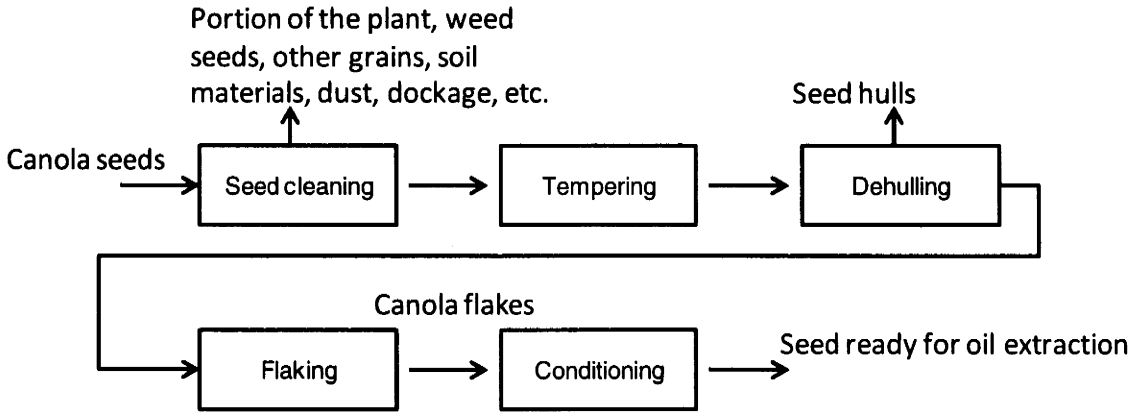

Figure 4. Canola seed pretreatment prior to oil extraction.

The pretreated seed is then ready for oil extraction which can be achieved through extraction techniques. Extraction can be categorized into three main types: mechanical extraction, solvent extraction, and the combination of mechanical and solvent extraction. The most common practice is to reduce seed oil content to less than 20% by screw presses or extrusion, followed by solvent-extraction to obtain the remaining oil. Compared with the screw press, extrusion better facilitates the next step of solvent extraction, since it produces a more porous canola cake (Pickard, 2001). The mechanical extraction alone typically obtains an oil recovery of 70% (D. Wiesenborn, personal communication, 2010), and combining mechanical with solvent extraction helps increase recovery to > 90%. Product oils extracted by different methods may vary in their composition and characteristics. Azadmard-Damirchi et al. (2010) found hexane extraction improved the Rancimat

oxidative stability (2.5 g, 110°C, and 20 L/h) of rapeseed oil to 2.5 h from 1 h by cold press, and tocopherols content was increased from 510 ppm to 596 ppm. They also found that 2 to 4 min microwave pretreatment of rapeseed tremendously improved the antioxidant content (811 to 924 ppm) and oxidative stability (5 to 8 h) of the resulting oil, probably due to the oilseed cell membrane breakdown by microwave pretreatment.

The crude canola oil is further processed to remove phospholipids (about 1.25%) by oil degumming. Additional refining can be completed by physical refining or alkaline refining. Depending on the uses of the resulting canola oil, further refining may be required, such as bleaching, winterization, and deodorization. The main reason for removing phospholipids and metallic pro-oxidants is that they are likely to form emulsions or sludge during processing or storage, therefore increasing processing cost and oil loss (Booth, 2004). The phosphorus content of crude crambe oil was reduced from 201 to 129 mg/kg using a combination of degumming (using citric acid solution) and neutralization (alkali refining) with an oil recovery of nearly 96% (Vargas-Lopez et al., 1999). For rapeseed oil, degumming was reported to reduce phosphorus content to only 2 mg/kg (Vargas-Lopez et al., 1999).

Methods for Biodiesel Preparation

Canola oil can be modified to be an alternative to diesel fuel with reduced viscosity and appropriate fuel properties through several techniques such as blending with petro diesel, incorporating into a microemulsion, pyrolysis and TE. The most commonly used method is TE, which converts TG into its methyl ester or ethyl esters (Atadashi et al., 2010). The TE method can further be classified into different types according to conditions used: conventional alkaline-, conventional acid-, metal oxides-, solid acid-, enzyme-, or

whole cell-catalyzed TE, co-solvent monophasic TE, supercritical methanol TE, and *in situ* TE (Haas and Foglia, 2005; Serio et al., 2008; He et al., 2008; Ranganathan, 2008; Kansedo et al., 2009). The supercritical methanol TE process was first demonstrated by Saka and Kusdiana (2001) to convert rapeseed oil to methyl esters. The result showed the biodiesel yield could reach 95% in 240 s without methoxide catalyst.

Given the good potential for use in a canola breeding program, conventional alkaline- and *in situ* alkaline-catalyzed TE methods are discussed in this literature review section.

Conventional alkaline TE

The TE reaction, usually with TG and methanol as reagents, sequentially converts the glycerin part of the TG to diglycerides (DG), monoglycerides (MG), and glycerin, with fatty acid methyl ester (FAME; biodiesel) formation in each step (Figure 5). Sodium hydroxide and potassium hydroxide are the most commonly used alkaline homogenous catalysts. When sodium or potassium hydroxides are mixed with methanol, methoxides form and work as the real catalysts to attack the carbonyl carbon atom of the TG molecule during TE (Serio et al., 2008).

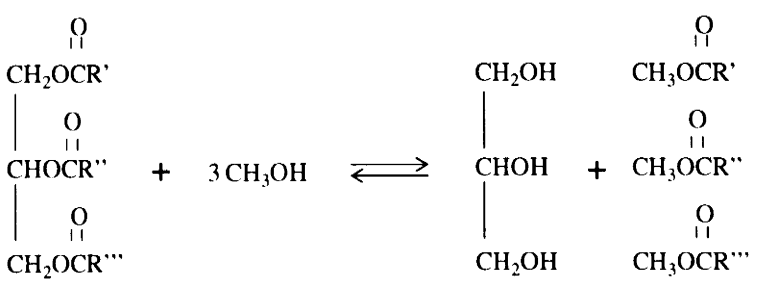

Figure 5. Schematic of transesterification (TE) of TG with methanol. R', R'', and R''' are long-chain saturated or unsaturated hydrocarbons.

After catalyst preparation, a typical alkaline TE biodiesel production process (referred to here as "conventional TE") beginning with refined canola oil (refer to the *Oil processing* section) includes the following steps: TE, glycerin separation, methanol removal, water wash, and vacuum drying (Figure 6). Small plants tend to use batch reactors, while larger plants (>4 million L/yr) favor continuous stirred-tank reactors (Haas and Foglia, 2005). Canola oil, catalyst, and methanol are combined and mixed, allowing the reaction to last for 1 h. The methanol is not added at the stoichiometric molar ratio of 3:1 (methanol to TG), but at the ratio of 6:1 or higher. When the reaction is completed, the post-reaction mixture is allowed to settle for 30 min to separate the glycerin layer from the biodiesel layer. After that, the excess methanol should be removed by heating biodiesel at about 65°C. The crude biodiesel is then subjected to water wash in order to remove remaining catalyst, soap, salts, methanol, and glycerin. A vacuum drying step is followed to remove water from the washed biodiesel. The refined biodiesel is then ready for fuel use directly or as a blending agent in diesel engine.

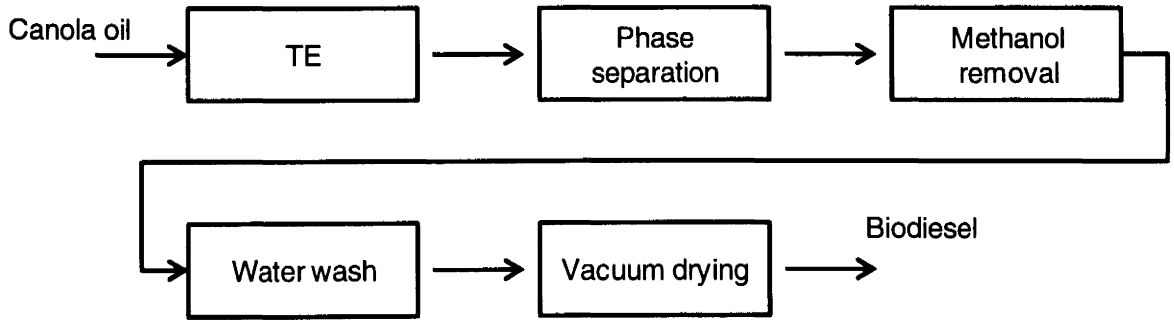

Figure 6. A typical alkaline TE process for biodiesel production.

When feedstock oils containing high levels of FFA are directly subjected to alkaline TE, the reaction encounters problems due to neutralization of FFA to soap by the base

catalyst. Pretreatment is thus required to reduce FFA concentration. When moisture is present in feedstock oils, it causes the hydrolysis of esters into alcohol and undesired FFA, thus impacting the TE process by increasing the level of soap. Therefore, when feedstock canola oil happens to have high FFA or moisture, more pretreatment steps or a modified TE process should be employed to achieve high biodiesel yield and good quality. For example, an acid esterification could be used to reduce the FFA content in feedstock oil, and then the alkaline TE is used (Ramadhas et al., 2005).

***In situ* TE**

The *in situ* TE method, illustrated in Figure 7, directly converts seed lipids to biodiesel without the prior oil extraction and refining steps which are used in the conventional alkaline TE. This method has already been applied for some oilseeds, such as soybean and cottonseed, on a scale of less than 30 g of oilseeds with acceptable yields (Siler-Marinkovic and Tomasevic, 1998; Haas et al., 2004; Georgogianni et al., 2008; Qian et al., 2008). It was first evaluated with acid catalysts for sunflower biodiesel preparation with 20% higher yield than the conventional TE (Harrington and D'Arcy-Evans, 1985a). The authors explored this method for the following reasons: 1) Biodiesel yield could be increased by subjecting the whole sunflower seed (including seed hull itself, which accounts for 40% of the total seed) to methanol as a solvent, and the lipid loss caused by dehulling could be eliminated; 2) Use of hexane, which is a dangerous and expensive solvent, could be eliminated; and 3) The post-reacted sunflower meal might show better digestibility.

Reaction parameters of the *in situ* TE impacting the resulting biodiesel yield and quality are complex and worth further research. These parameters include seed lipid

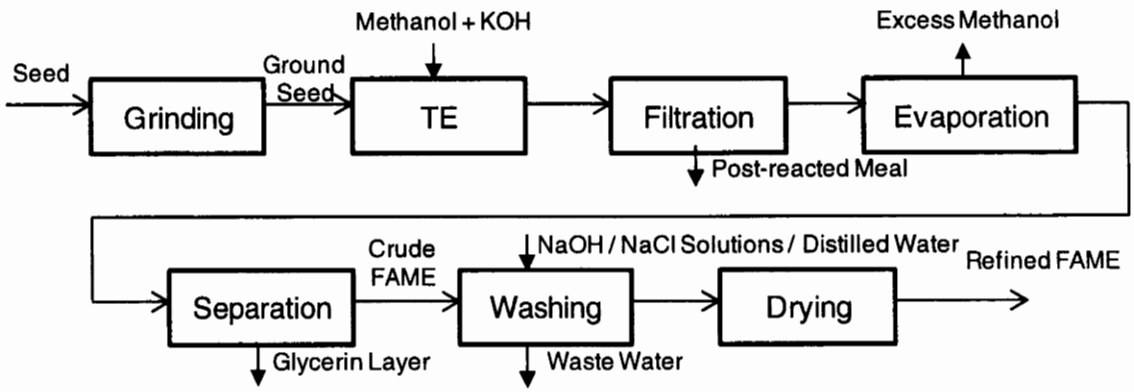

Figure 7. Process flow scheme of the *in situ* TE for biodiesel production from oilseeds.

content, co-solvent use, feedstock grinding method, seed moisture content, catalyst and alcohol type, molar ratio of catalyst to alcohol to TG, reaction temperature and time, agitation method, reactor type, and biodiesel refining method. Table 2 lists reaction variables, yield, and evaluated biodiesel qualities of methods reported in the scientific literature. The original oil content of feedstocks reported ranged from 20% to 40%. Whatever agitation method, catalyst and alcohol type, reactor type, and refining method chosen, biodiesel yields of *in situ* TE are generally very high (> 90%) on a basis of the total mass of crop seed or feedstock. Utilization of co-solvent in the reaction mixture helps decrease the molar ratio of alcohol to lipid (about 150:1) and reaction time but still achieves high yield. This is because co-solvents promote the lipid extraction from seeds, and accelerate the reaction by improving the mass transfer between oil and methanol (Zeng et al., 2009). In order to obtain high yield, acid *in situ* TE required about 3 h, while the alkaline-catalyzed reaction reduced the reaction time to 0.5 h or less. However, the reaction scale of these *in situ* TE studies was 5 to 25 g of seed, which resulted in no more than 9 g biodiesel. Quality evaluations would be severely limited by this low biodiesel amount. These studies did not report relevant data to determine suitability of their methods for

rapidly evaluating many samples, such as the number of samples processed at one time, since their focus was on biodiesel yield. Recently, researchers simultaneously extracted lipid from oil-bearing feedstock, and converted it to biodiesel using the supercritical methanol process (Lim et al., 2010; Patil et al., 2010). A major obstacle for using the supercritical methanol process is the high temperature and pressure required during TE, which should be higher than 240°C and 7.9 MPa, respectively.

In situ TE rapidly produces biodiesel with high yield (>90% seed lipids), eliminates the oil extraction and oil refining, and is suitable for biodiesel production with a high throughput in one reaction batch. Optimizing the reaction parameters of *in situ* TE and evaluating the corresponding biodiesel qualities are desirable. To date, challenges still exist in this method, such as scaling up the method to produce sufficient sample, and simultaneously processing multiple samples per batch to save operator time (see the *Paper 1*); however, due to its advantages mentioned above, this method might be integrated with a canola breeding program to efficiently provide biodiesel samples. It has potential use as an integral part of higher throughput techniques for canola line evaluation.

Cold Weather Performance (CWP) of Biodiesel

The ASTM D 6751-09 specifications (ASTM, 2009) should be met before biodiesel is sold in the United States, or the EN 14214 specifications in Europe. Several important biodiesel quality traits are as follows: methanol content, moisture content, kinematic viscosity, acid number, cetane number, cloud point, total and free glycerol, cold soak filterability, and oxidation stability. Most of these qualities should be acceptable if the biodiesel production process is set up well; however, some properties are more feedstock-associated, such as the cold soak filterability (indicating the CWP of biodiesel) and

Table 2. A summary of literature reports of *in situ* TE research (continued next page).

Feedstock (MW [a])	Oil wt. %	CT [b]	CST:oil [c]	Al [d]	Preparation	MC [e] %	Al: oil: CT [f]	Opt. ratio [g]	References [h]
SF (876)	45.63	KOH NaOH	DEM/ 57.85:1	M	Coffee grinder	4.63	24:1:0.2 to 261.8:1:2.4	101.39:1:0.5	1
SB (914)	23.9	NaOH	None	M	Flaked	7.4	A series for each T	543:1:2.0 and 226:1:1.6	2
SB (914)	20.5	NaOH	None	M	Flaked	2.6 and 0	A series for each MC	370:1:1.5 and 240:1:0.98	3
CS (858)	22	NaOH	None	M&E	Macerated	6.1	1.0, 1.5, 2.0% of NaOH	965:1:0.625	4
CS (858)	31.6	NaOH	Petroleum ether	M	Grinder	1.9 and 8.7	85:1:0.17 to 170:1:0.69	135:1:0.55	5
SF (875)	37.8	Sulfuric acid	None	M&E	Macerated	6.2	430:1:13	NA	6
SF (875)	40	Sulfuric acid	None	M	Coarse sand	4-5	430:1:13	NA	7
SF (875)	40	NaOH	None	M&E	Macerated	5.6	1.0, 1.5, and 2.0% of NaOH	541:1:0.35	8
SB (875)	23.6	Sulfuric acid	CO_2 gas-expanded	M	Flaked	1.6	108:1:2.8 to 217:1:17	217:1:5.6	9
RB (872)	17	NaOH (2-stage)	None	M	As it was	4	Not clear	2115:1:21	10

[a] MW: estimated molecular weight of feedstock oil; Feedstock: SF, sunflower seeds; SB, soybean; CS, cottonseed; and RB, rice bran.
[b] CT: catalyst (the real catalyst was corresponding methoxide when base methanol solution was used).
[c] (CST:oil): the molar ratio of co-solvent to triglycerides; DEM: diethoxymethane.
[d] Al: alcohol, either methanol (M) or ethanol (E).
[e] MC: moisture content.
[f] Molar ratio of alcohol to TG to catalyst.
[g] t: reaction time; opt. t: optimal reaction time.
[h] References:1, Zeng et al., 2009; 2, Haas et al., 2004; 3, Haas and Scott, 2007; 4, Georgogianni et al., 2008a; 5, Qian et al., 2008; 6, Harrington and D'Arcy-Evans, 1985a; 7, Harrington and D'Arcy-Evans, 1985b; 8, Georgogianni et al., 2008b; 9, Wyatt and Haas, 2009; and 10, Shiu et al., 2010.

Table 2. A summary of literature reports of *in situ* TE research (continued from previous page).

Feedstock (MW [a])	t [b]/Opt. t	T [c]/opt. T (°C)	Size (g)	Mixing	Reflux [d]	Refining	Yield (%)	Quality	References [e]
SF (876)	2-16 min/13 min	20-65/20	20	MS, 150 rpm	Y	*n*-hexane extraction	97.3	FFA	1
SB (914)	8 h	23, 60/NA	5	OS	N	Hexane extraction	93	FFA; TG	2
SB (914)	4-10 h/16 and 10 h	23/NA	5	OS	N	Water wash	97 and 100	FFA; TG	3
CS (858)	10 min to 4 h	60 (for M); 80 (for E)	20	MS, 600 rpm; US, 24KHz	Y	Petroleum ether extraction	97	Kinetics research	4
CS (858)	1 to 5 h/3 h	30-65/40	25	MS	Y	Petroleum ether	98	NA	5
SF (875)	3 to 4 h	NA	20	NA	Y	Petroleum ether	100	NA	6
SF (875)	4 h	NA	20	NA	Y	Petroleum ether	100	CP	7
SF (875)	5 min to 4 h	60 (for M); 80 (for E)	20	MS, 600 rpm; US, 24KHz	Y	Petroleum ether	98	NA	8
SB (875)	3 to 10 h/10 h	24-121/121 (Pressure=7.38 MPa)	22.5	MS, 300 rpm	N	Centrifuge	88.3 or lower	FFA; TG	9
RB (872)	0 to 2.5 h/0.5 h	NA/60	10	MS, 600 rpm	Y	Hexane extraction	92 or lower	NA	10

[a] MW: estimated molecular weight of feedstock oil; Feedstock: SF, sunflower seeds; SB, soybean; CS, cottonseed; and RB, rice bran.

[b] T: reaction temperature; opt. T: optimal reaction temperature.

[c] Three mixing methods were used: mechanical stirring(MS), orbital shaking (OS), and ultrasonic (US).

[d] Some experiments employed reflux system (Y) and other not (N).

[e] The same references as in the first part of the Table 2.

oxidative stability (associated with FA profile and some other minor components). These properties should be monitored in oilseed breeding programs.

The CWP of biodiesel plays an important role for biodiesel distributed and consumed in cold winter areas. When used in cold weather conditions, biodiesel may cause problems relating to engine performance, such as fuel line and fuel filter plugging (Dunn, 2009).

Some quality standards have been consequently proposed for determining CWP of biodiesel both in ASTM D 6751 and EN 14214 (Table 3). The ASTM D 6751 requires the cloud point to be reported. The cold filter plugging point is included in EN 14214 specifications. The cold soak filtration test (CSFT; ASTM D 7501-09b), a method determining the fuel filter blocking potential of biodiesel, was instituted by the ASTM (2009) and included in the most recent ASTM D 6751 specifications as the "cold soak filterability (CSF)". The ASTM standard requires the cold soak filterability of neat (without blending) biodiesel be no greater than 360 s for blending up to B20. In situations where the temperature could be below -12°C, the cold soak filterability limit is 200 s. The CSFT uses 300 mL biodiesel for each measurement. Studies using Fourier Transform Infrared Spectroscopy (FT-IR) to determine the filterability are on-going, because FT-IR is rapid and the sample volume requirement for each test is small (Stefl et al., 2009).

Biodiesel components have different melting points, and the higher melting point components would precipitate out of the liquid phase and plug fuel filters at correspondingly higher temperatures. The fatty acid profile of the original oil and presence of some trace contaminants contribute to biodiesel's CWP as well. After biodiesel is subjected to low temperatures, part of the saturated MG and FAME crystallize, and may

Table 3. Tests representing the cold weather performance of biodiesel.

Parameter	Units	Limit	Brief description	Method
Kinematic viscosity	mm^2/s	1.9 to 6.0	Proportional to time period necessary for biodiesel to flow by gravity through a certain diameter capillary at 40°C	ASTM D 445
Cloud point	°C	Report	Temperature where haziness (1^{st} wax crystallization) is observed	ASTM D 2500
Pour point	°C	N	Lowest temperature where free surface movement is observed	ASTM D 97
LTFT	°C	N	Lowest temperature at which a 180 mL sample passes through 17 μm wire mesh under 0.197 atm vacuum within 60 s	ASTM D 4539
CFPP	°C	N	Lowest temperature at which a 20 mL sample passes through 45 μm wire mesh under 0.0194 atm vacuum within 60 s	ASTM D 6371
CSFT	s	360 (or 120)	Time necessary for 300 mL biodiesel to be filtered under 21to 25 in Hg vacuum at 25°C	ASTM D 7501

LTFT: low temperature flow test.
CFPP: cold filter plugging point.
CSFT: cold soak filtration test.
Limit: indicating limits in ASTM D 6751 of specification for B100.
N: no requirement in ASTM D 6751.

remain solid when the temperature slightly increases (Dunn, 2009). The TE procedure sequentially converts TG to DG, MG (TG, DG, and MG are defined on page 14), and finally glycerin. These intermediates become trace contaminants if they are not totally removed in the refining step, especially DG and MG since they are insoluble in water. For example, monopalmitin and monostearin, with melting point of 77 and 82°C respectively, do not readily return to liquid again after precipitating from biodiesel liquid. There are also some other trace contaminants which tend to form precipitates, such as soaps and steryl glucosides; the latter is an important issue in soybean biodiesel (Dunn, 2009). When a diesel engine runs with biodiesel of poor CWP, fuel line and engine filter clogging as well as injector deposits may happen, and these engine problems may stop operation. Factors impacting biodiesel CWP are summarized in Table 4.

Table 4. A summary of factors affecting the cold weather performance of biodiesel.

Category	Factors	Effect on cold weather performance (CWP)
Fatty acids	Chain length	Long carbon chains impair the CWP
	Saturation	Saturated carbon chains decrease the CWP
	Trans-fatty acids	*Trans* isomers decrease the CWP
Trace containments	Bound glycerides Glycerin Steryl glucosides Soap Moisture Waxes Dimer of methyl esters	Form sediment and act as wax crystal nucleators and may interact with other components to increase sedimentation and wax crystallization
Others	Crop growing Thermal history Blending Processing	Influence the crystallization process, suspension of sediment, and content of other factors

References for this table are as follows: Mittelbach and Gangl, 2001; Dunn, 2005; Lee et al., 2007; Bondioli et al., 2008; Selvidge, 2008; Dunn, 2009; and Moser, 2009.
Note that *cis* and *trans* isomers indicate that functional groups are on the same side and the other side, respectively.

Other Quality Traits of Biodiesel

Besides cold weather performance, there are some other important quality traits associated with feedstock lipid quality. A canola breeding program should be able to screen out unacceptable breeding lines; this requires evaluation of potentially hundreds or even thousands of samples per year representing different genotypes and growing locations, which in turn requires rapid methods for evaluating biodiesel quality. These quality traits include kinematic viscosity, free fatty acid content, total and free glycerin, and oxidative stability.

Oxidative stability

Oxidative stability of biodiesel is an indicator of biodiesel storage stability (Knothe, 2005). It may be evaluated by the Rancimat test, and the result is presented as oxidative

stability index (OSI). This method is almost the same with the AOCS Cd 12b-92 method for determining oil stability index, except the amount of liquid used (5 g). The Rancimat test exposes 3 g (both in EN 14214 and ASTM D 6751 specifications) biodiesel sample to aeration at 110°C. The volatiles produced by oxidation are carried to a water trap, and increase water conductivity. After an initial induction period in which conductivity remains uniformly low, oxidation accelerates and becomes very rapid with an increasing amount of volatile components formed by biodiesel degradation. The OSI measures the induction period by plotting conductivity against time and calculating the maximum of the second derivative.

Methyl esters of saturated fatty acids have excellent oxidative stability (Table 5), but poor cold weather performance. In contrast, methyl esters of polyunsaturated fatty acids are sensitive to oxidation, but have excellent cold weather performance when not degraded (Dunn, 2005). Oleic acid, the predominate fatty acid in canola oil (Table 1), provides a balance between these two properties, as it has intermediate values for both. Compared to biodiesel derived from soybean oil and sunflower oil, the fatty acid profile of canola yields biodiesel with satisfactory cold weather performance and oxidative stability (Table 6).

Table 5. Oxidative stability index (OSI) of several pure fatty acid methyl esters (Moser, 2009).

FAME	**Methyl palmitate**	**Methyl stearate**	**Methyl oleate**	**Methyl linoleate**	**Methyl linolenate**
OSI (h)	> 40	> 40	2.5	1.0	0.2

These OSI were tested according to EN 14112: 3 g sample at 110°C with air stream 10 L/h.

Table 6. OSI and cloud point value of methyl ester from different feedstocks (Moser, 2009).

Feedstock	Soybean	Sunflower	Canola
Cloud point (°C)	1±1	5±1	0±1
OSI (h)	5.0±0.1	6.2±0.1	6.4±0.1

References

ASTM. 2009. Standard specification for biodiesel fuel blend stock (B100) for distillate fuels, designation D 6751-09. West Conshohocken, Pa.: American Society for Testing and Materials.

Atadashi, I.M., M.K. Aroua, and A.A. Aziz. 2010. High quality biodiesel and its diesel engine application: A review. *Renew. Sust. Energ. Rev.* 14(7): 1999-2008.

Azadmard-Damirchi, S., F. Habibi-Nodeh, J. Hesari, M. Nemati, and B.F. Achachlouei. 2010. Effect of pretreatment with microwaves on oxidative stability and nutraceuticals content of oil from rapeseed. *Food Chem.* 121(4): 1211-1215.

Bondioli, P., N. Cortesi, and C. Mariani. 2008. Identification and quantification of steryl glucosides in biodiesel. *Eur. J. Lipid Sci. Technol.* 110(2): 120-126.

Booth, E.J., and F.D. Gunstone. 2004. Rapeseeds and rapeseed oil: agronomy production, and trade. In *Rapeseed and Canola Oil: Production, Processing, Properties and Uses*, 1-16. F.D. Gunstone, ed. Boca Raton, Fla.: CRC Press.

Booth, E.J. 2004. Extraction and refining. In *Rapeseed and Canola Oil: Production, Processing, Properties and Uses*, 17-36. F.D. Gunstone, ed. Boca Raton, Fla.: CRC Press.

Botha, I., R. Mailer, and K. Robards. 2000. Evaluation of the role of saturated fatty acids in sedimenting canola oils. *J. Am. Oil Chem. Soc.* 77(4): 429-435.

Duke, J.C., F.M. Epplin, J.D. Vitale, and T.F. Peeper. 2009. Canola-wheat rotation versus continuous wheat for the Southern Plains. Atlanta: Southern Agricultural Economics Association Annual Meeting, Atlanta, Georgia, January 31-February 3.

Dunn, R. O. 2005. Cold weather properties and performance of biodiesel. In *The Biodiesel Handbook*, 83-121. G. Knothe, J. Krahl, and J. Van Gerpen, eds. 1st Ed. Champaign, Ill: AOCS Press.

Dunn, R. O. 2009. Effects of minor constituents on cold flow properties and performance of biodiesel. *Prog. Energy Comb. Sci.* 35(6): 481-489.

Georgogianni, K.G., M.G. Kontominas, P.J. Pomonis, D. Avlonitis, and V. Gergis. 2008a. Conventional and in situ transesterification of sunflower seed oil for the production of biodiesel. *Fuel Process. Technol.* 89(5): 503-509.

Georgogianni, K.G., M.G. Kontominas, P.J. Pomonis, D. Avlonitis, and V. Gergis. 2008b. Alkaline conventional and in situ transesterification of cottonseed oil for the production of biodiesel. *Energy Fuels* 22(3): 2110-2115.

Haas, M.J., and T.A. Foglia. 2005. Alternate feedstocks and technologies for biodiesel production. In *The Biodiesel Handbook*, 42-61. G. Knothe, J. Krahl, and J. Van Gerpen, eds. 1st Ed. Champaign, Ill: AOCS Press.

Haas, M.J., and K.M. Scott. 2007. Moisture removal substantially improves the efficiency of in situ biodiesel production from soybean. *J. Am. Oil Chem. Soc.* 84(2): 197-204.

Haas, M.J., K.M. Scott, W.N. Marmer, and T.A. Foglia. 2004. In situ alkaline transesterification: An effective method for the production of fatty acid esters from vegetable oils. *J. Am. Oil Chem. Soc.* 81(1): 83-89.

Harrington, K.J., and C. D'Arcy-Evans. 1985a. Transesterification in situ of sunflower seed oil. *Ind. Eng. Chem. Prod. Res. Dev.* 24(2): 314-318.

Harrington, K.J., and C. D'Arcy-Evans. 1985b. A comparison of conventional and in situ methods of transesterification of seed oil from a series of sunflower cultivars. *J. Am. Oil Chem. Soc.* 62(6): 1009-1013.

He, H., T. Wang, and S. Zhu. 2008. Continuous production of biodiesel fuel from vegetable oil using supercritical methanol process. *Fuel* 86(3): 442-447.

Ikebudu, JA, S Sokhansanj, R.T. Tyler, B.J. Milne, and N.S. Thakor. 2000. Grain conditioning for dehulling of canola. *Can. Agric. Eng.* 42 (1): 27-32.

Kansedo, J., K.T. Lee, and S. Bhatia. 2009. Biodiesel production from palm oil via heterogeneous transesterification. *Biomass Bioenergy* 33(2): 271-276.

Kulkarni, M.G., A.K. Dalai, and N.N. Bakhshi. 2006. Utilization of green seed canola oil for biodiesel production. *J. Chem. Technol. Biotechnol.* 81(12): 1886-1893.

Knothe, G. 2005. Literature Overview. In *The Biodiesel Handbook*, 122-126. G. Knothe, J. Krahl, and J. Van Gerpen, eds. 1st Ed. Champaign, Ill: AOCS Press.

Lee, I., L. M. Pfalzgraf, G. B. Poppe, E. Powers, and T. Haines. 2007. The role of sterol glucosides on filter plugging. *Biodiesel Magazine*. Available at: www.biodieselmagazine.com. Accessed 20 December 2009.

Lim, S., S.S. Hoong, L.K. Teong, and S. Bhatia. 2010. Supercritical fluid reactive extraction of Jatropha curcas L. seeds with methanol: A novel biodiesel production method. *Biores. Technol.* 101(18): 7169-7172.

Mittelbach, M., and S. Gangl. 2001. Long storage stability of biodiesel made from rapeseed and used frying oil. *J. Am. Oil Chem. Soc.* 78 (6): 573-577.

Moser, B. R. 2009. Biodiesel production, properties, and feedstocks. *In Vitro Cell. Dev. Biol.-Plant* 45 (3): 229-266.

O'Brien, R.D. 2009. Raw Materials. In *Fats and Oils: Formulating and Processing for Applications*, 1-72. R.D. O'Brien, ed. 3rd Ed. Boca Raton, Fla.: CRC Press.

Patil, P.D., V.G. Gude, A. Mannarswamy, S. Deng, P. Cooke, S. Munson-McGee, I. Rhodes, P. Lammers, and N. Nirmalakhandan. 2010. Optimization of direct conversion of wet algae to biodiesel under supercritical methanol conditions. *Biores. Technol.* 102(1): 118-122.

Pickard, M.D. 2001. Canola Meal Feed Industry Guide, Section I, processing of canola seed for quality meal. Winnipeg, Canada: Canola Council of Canada.

Qian, J.F., F. Wang, S. Liu, and Z. Yun. 2008. In situ alkaline transesterification of cottonseed oil for production of biodiesel and nontoxic cottonseed meal. *Biores. Technol.* 99(18): 9009-9012.

Ramadhas, A.S., S. Jayaraj, and C. Muraleedharan. 2005. Biodiesel production from high FFA rubber seed oil. *Fuel* 84(4): 335-340.

Ranganathan, S.V., S.L. Narasimhan, and K. Muthukumar. 2008. An overview of enzymatic production of biodiesel. *Biores. Technol.* 99(10): 3975-3981.

Ratnayake, W.M.N., and J.K. Daun. 2004. Chemical composition of canola and rapeseed oils. In *Rapeseed and Canola Oil: Production, Processing, Properties and Uses*, 37-78. F.D. Gunstone, ed. Boca Raton, Fla.: CRC Press.

Saka, S., and D. Kusdiana. 2001. Biodiesel fuel from rapeseed oil as prepared in supercritical methanol. *Fuel* 80(2): 225-231.

Selvidge, C. 2008. Biodiesel fuel blend, US Patent 11/674, 887.

Serio, M.D., R. Tesser, P. Lu, and E. Santacesaria. 2008. Heterogeneous Catalysts for Biodiesel Production. *Energy Fuels* 22(1): 207-217.

Siler-Marinkovic, S., and A. Tomasevic. 1998. Transesterification of sunflower oil in situ. *Fuel* 77(12): 1289-1391.

Stefl, B., N. Wang, and C.-H. Tseng. New advances in the use of fourier transform infrared spectroscopy for the analysis of biodiesel. Available at: http://www2.qta.com. Accessed 20 Jan 2010.

Tostenson, K., D. Wiesenborn, D. Haagenson, V. Hofman, K. McKay, B. Jenks, and S. Halley. 2007. Oil and biodiesel from canola having a high content of green seed. ASABE Paper No. RRV-07110. Fargo, N.D.: ASABE/CSBE North-Central Intersectional Conference.

USDA. 2009. North Dakota Canola Production Statistics: 2008. National Agricultural Statistics Database. Washington, D.C.: USDA National Agriculture Statistics Service. Available at: http://www.nass.usda.gov. Accessed 5 Jan 2010.

Vargas-Lopez, J.M., D. Wiesenborn, K. Tostenson, and L. Cihacek, 1999. Processing of crambe for oil and isolation of erucic acid. *J. Am. Oil Chem. Soc.* 76(7): 801-809.

Viola, F., S. Oliaro, A. Binello, and G. Cravotto. 2008. Policosanol: updating and perspectives. *Mediterr. J. Nutr. Metab.* 1(2): 77-83.

Walker, K. 2004. Non-food use. In *Rapeseed and Canola Oil: Production, Processing, Properties and Uses*, 1-16. F.D. Gunstone, ed. Boca Raton, Fla.: CRC Press.

Wyatt, V.T., and M. J. Haas. 2009. Production of Fatty Acid Methyl Esters via the In Situ Transesterification of Soybean Oil in Carbon Dioxide-Expanded Methanol. *J. Am. Oil Chem. Soc.* 86(10): 1009-1016.

Zeng, J., X. Wang, B. Zhao, J. Sun, and Y. Wang. 2009. Rapid in situ transesterification of sunflower oil. *Ind. Eng. Chem. Res.* 48(2): 850-856.

PAPER 1: YIELD AND CHARACTERISTICS OF CANOLA BIODIESEL PREPARED THROUGH CONVENTIONAL AND IN SITU TRANSESTERIFICATION WITH VARIOUS SEED MOISTURE CONTENTS

Paper 1 is an edited and revised version of a paper presented at the ASABE Annual International Meeting in Reno, NV, June 21 – 24, 2009. Authors: Hongjian Lin, Darrin Haagenson, Rachel Brudvik, and Dennis Wiesenborn. Title: Influence of Seeds Moisture on in situ Alkaline Transesterification of Canola Seeds for Biodiesel Production. Paper number: 096339. Hongjian Lin, the author of this thesis, is the first author of Paper 1. He designed and conducted the experiment in this paper and also wrote Paper 1, which was edited by the other co-authors.

ABSTRACT

Recent market interest in producing biodiesel from canola seed has prompted research on biodiesel preparation techniques and rapid screening methods for biodiesel quality traits. *In situ* alkaline transesterification (iTE) has been reported as an efficient method for biodiesel preparation from several oilseed crops such as cottonseed, sunflower and soybean. The primary objective of this study was to determine the effect of canola seed moisture content (MC, ranging from 0% to 6.7%) on iTE biodiesel yield, and to compare the efficiency of the iTE and conventional TE (CTE) methods as well as their biodiesel properties. Canola seeds pooled from small amounts of various genotypes were converted to biodiesel with different MC treatments through the two preparation methods. Several properties were examined, including kinematic viscosity, oxidative stability, and free fatty acid, moisture and total glycerin content. Results showed that seed moisture content had a significant effect on iTE biodiesel yield ($p < 0.001$). Results also showed significant differences ($p < 0.05$) in some properties (moisture content, total glycerin, and oxidative stability) between different biodiesel preparation methods. Total glycerin of iTE samples was much reduced, and other properties were generally satisfactory with the ASTM D 6751 specifications. This study shows good potential to implement the iTE method for incorporation with a canola seed breeding program for screening biodiesel use.

INTRODUCTION

Biodiesel (fatty acid methyl esters, or FAME) derived from vegetable oils is an alternative to diesel fuels with satisfactory use in diesel engines as B100 or blending agents (Knothe, 2005a; Meher et al., 2006). The primary chemical reaction converting vegetable oils (mainly triglycerides, or TG) into biodiesel is transesterification (TE). Biodiesel in industry is generally produced by alkaline TE from refined vegetable oils (Fukuda et al., 2001); the method is referred to as conventional TE (CTE) in this article. CTE is performed with the presence of methanol and alkaline catalysts such as sodium hydroxide, potassium hydroxide or their methoxides, resulting in FAME and the by-product glycerin.

In CTE, the oil is extracted and/or refined prior to TE. *In situ* TE (iTE) is an alternative approach that converts seed lipids to FAME without a separate oil extraction step. The idea of iTE was first tested on sunflower using an acid catalyst (Harrington and D'Arcy-Evans, 1985a). This method was later evaluated and optimized for yield with various feedstocks, such as soybean and cottonseed (Siler-Marinkovic and Tomasevic, 1998; Haas et al., 2004; Georgogianni et al., 2008; Qian et al., 2008). Harrington and D'Arcy-Evans (1985b) compared cloud point of biodiesel prepared through iTE and CTE, and found that iTE resulted in desired, slightly lower cloud point. Soy biodiesel, prepared through the iTE method, met the ASTM D 6751-09 specifications (ASTM, 2009; Haas and Scott, 2007).

Canola (*Brassica napus* L.) is an important potential biodiesel feedstock due to its high yield per acre and high oil content (40 to 50%). The high monounsaturated fatty acids content (63%) and low saturated fatty acids (< 7%) (Ratnayake and Daun, 2004) of canola may yield biodiesel with improved oxidative stability and cold weather performance,

compared with biodiesel derived from soybean or animal fats, respectively (Dunn, 2005; Knothe, 2008). North Dakota canola production accounts for approximately 90% of the US national acreage with 360,000 ha harvested in 2008 (USDA, 2008). As demands for canola oil for both food and fuel increase, canola breeding efforts are ongoing to identify elite lines that perform well across contrasting growing environments. However, the relationships of genotype and growing environment with canola fatty acid composition, other trace components, and biodiesel properties are not clearly understood.

Objectives

Adapting an iTE method to canola may provide a high throughput screening tool for assessing genotype or environment effects on canola biodiesel. The primary objective of this study was to determine the effect of canola seed moisture content on iTE biodiesel yield, and to compare the efficiency of the iTE and conventional TE (CTE) methods as well as their biodiesel properties. Canola seeds obtained from two growing locations in North Dakota were evaluated for iTE yields at three seed MCs ranging from 0% to 6.7%. Important biodiesel properties, including kinematic viscosity, acid value, moisture content, total glycerin, and oxidative stability, were compared among samples prepared through the iTE and CTE methods.

MATERIALS AND METHODS

Materials

Canola seeds of bulked varieties (*Brassica napus* L.) grown in Williston (Wil.) and Prosper (Pros.), ND in 2007, were obtained. The two growing locations were chosen due to the variability in available precipitation and temperature. Prosper, on average, was 1°C cooler and received approximately twice the precipitation of Williston in the growing season (NDAWN, 2007). Canola seed was cleaned according to USDA-GIPSA method (2004). All reagents were of analytic reagent grade and purchased from EMD Chemicals (Gibbstown, NJ).

In Situ Transesterification (iTE)

The iTE for canola seed was adapted from a soybean *in situ* TE protocol reported previously by Haas and Scott (2007), with the process flowchart illustrated in Figure 8.

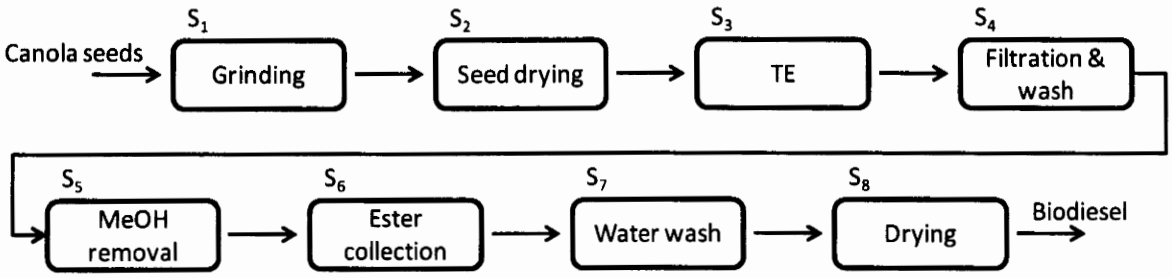

Figure 8. Flowchart for *in situ* TE.

Seed preparation

Canola seed (2 kg) with seed moisture from 4.1 to 4.6% (dry basis) was ground into flour with a coffee grinder (setting 3, Model DMB-8, Cuisinart, East Windsor, NJ). The resulting flour particle size was such that 86.5% and 31.0 wt. % passed through #20 and #50 mesh sieves (ASTM E-11), respectively. Canola flour was dried at 70°C overnight in a

gravity convection oven (18EG, Precision Scientific, Inc., Chicago, IL), and then subjected to different MC treatments by adding calculated volumes of distilled water (see *Experiment Design* section). The conditioned seed flour was sealed in polyethylene bags and stored at 4°C for 1 wk to achieve moisture equilibrium. In order to obtain accurate MC values, all sample MCs were confirmed gravimetrically prior to the initiation of the iTE.

Reaction conditions and washing

Canola flour (about 100 g) of 40 g oil-equivalent was transferred to a 1-L Erlenmeyer flask. Calculated volume of methanolic KOH was added to canola flour at the molar ratio of 350:1:1.17 of methanol/oil/KOH (or as the following mass: 508 g methanol, 40 g oil-equivalent (about 100 g canola flour), and 3.0 g KOH). TE was carried out at 47°C in a shaker water bath at 575 rpm for 16 h. After completion of the reaction, the samples were vacuum filtered through a Whatman #4 filter at 30 kPa. Post-reacted canola meal was washed three times each with 60 mL methanol, and the methanol washes were combined with the filtrates. For the first replicate samples, methanol in the pooled liquid was removed by a rotary evaporator (RE-111, Buchi, New Castle, DE) at 60°C and 75 kPa. For the second and third replicate samples, the pooled liquid was transferred to 13 cm × 33 cm aluminum baking pans, and the methanol was removed by vacuum oven drying (60°C, 75 kPa) for 24 h.

The dried crude FAME was weighed and washed sequentially with 1 vol of distilled water followed by 0.3 vol of 0.5*M* NaCl, 0.3 vol of 0.03*N* NaOH, 0.1 vol of 0.5*M* NaCl solution, and 1 vol distilled water, with centrifugation at 3500 × G for 15 min after each wash. Additional washes were employed for those samples having an excessive amount of emulsions. The washed samples were dried with the addition of 10% w/v anhydrous

sodium sulfate. The refined biodiesel samples were transferred to amber glass bottles and stored at room temperature until quality analysis.

Conventional Transesterification (CTE)

The CTE process is described in Tostenson et al. (2007), shown in Figure 9. Canola seed samples (2 kg) at 7% moisture were screw-pressed with a Komet Screw Press (S 87G, IBGMonforts, Germany) preheated to 70°C, equipped with a 6 mm opening die and an R8 screw operated at the speed of 20 rpm. Pressed oil was clarified and degummed with citric acid as described by Vargas-Lopez et al., 1999. The degummed oil samples were transesterified to biodiesel using the molar ratio of 6:1:0.2 of methanol/oil/KOH at 60°C for 1h with a magnetic stirrer at 500 rpm. The mixture was then allowed to settle for 30 min until the lower crude glycerin layer was drained from the separatory funnel by gravity. The upper crude biodiesel layer was heated (65°C, 17 kPa) in a 1-L flask for 30 min to remove the remaining methanol. It was then transferred back to the separatory funnel, and neutralized with 1 vol of 0.5% acetic acid solution, and then washed three times each with 1 vol of distilled water. The washed biodiesel was transferred to the 1-L flask, and dried for 30 min (95°C, 17 kPa). The refined biodiesel samples were transferred to amber glass bottles and stored at room temperature before quality analysis.

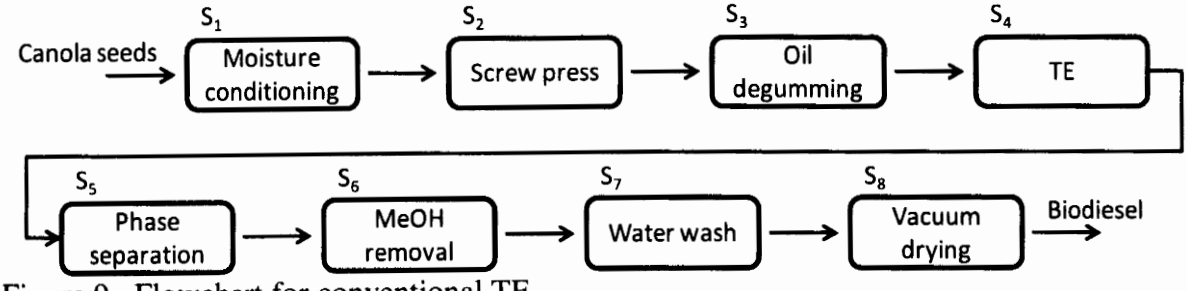

Figure 9. Flowchart for conventional TE.

Oil Content and Fatty Acid Profile

Canola oil content and principal fatty acid composition (wt. percentages of palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid) of intact seed samples from 2 locations were determined with an NIR spectrometer (DA 7200, Perten Instruments, Springfield, IL). Canola seeds (about 20 g) were transferred to a plastic cup of r = 30 mm and h = 10 mm. The seed spectrum was averaged from two samples with each subjected to 32 scans (accuracy better than 0.3 nm) using a ceramic reference. The spectrometer was programmed with prediction models for those two quality traits for the purpose of canola breeding and screening, combined with an outlier detection algorithm to make sure that the analyzed sample is within the spectral range of calibration.

Seed Moisture Content

Prior to the seed moisture (MC) analysis, canola seed was ground into flour (see *Seed Preparation* section). The MC was then determined gravimetrically at 120°C with an LJ16 moisture analyzer (Mettler Toledo, Inc., Columbus, OH).

Biodiesel Property Analysis

Kinematic viscosity (40°C) was determined using a capillary viscometer according to ASTM D 445. Biodiesel moisture content was determined using a Karl Fischer Coulometer (Model DL 32, Mettler Toledo, Inc., Columbus, OH). Free fatty acid (FFA) content, defined as "acid number" in ASTM specification, was determined by KOH titration, with phenolphthalein as an indicator (ASTM D 664). Oxidative stability was measured with an Omnion OSI (Omnion Inc., Rockland, MA) according to EN 14112. Total glycerin was quantified by SafTest kit according to its manufacturer's recommendation (MP Biomedical, Solon, OH).

Time Requirements of iTE and CTE

Processing steps for each batch of both iTE (assuming 10 reactors per batch) and CTE (assuming 8 reactors per batch) in the Pilot Plant laboratory were documented. The duration of each step was estimated after biodiesel preparation experiment. Biodiesel preparation consists of a set of sequential steps, S_k, $k \in \{1, 2, 3, \ldots, 8\}$ (Figure 8 and 9). T_k, O_k, and U_k denote the total time (h) required to complete S_k, operator time (h) for the step S_k, and unsupervised processing time (h) for the step S_k, respectively. Operator time is defined as the time taken to complete S_k by an operator. Unsupervised processing time is defined as the time running in a machine or an equipment to complete S_k without supervision of operators. Therefore, for each step, $T_k = O_k + U_k$. Operator time O_k typically includes a setup period, or SU_k (h), which is sometimes required before a regular operation.

Experiment Design

Impact of seed moisture content on iTE biodiesel yield

Canola flours from both Williston and Prosper in ND were subjected to 3 MC treatments: low (L) = 0 to 0.5%, moderate (M) = 4.1 to 4.6%, and high (H) = 6.6 to 6.7%. The amounts of moisture addition for these treatments were calculated by mass balance, and MC was checked just prior to the iTE. Thus, the experiment was arranged as a 2 × 3 full factorial design replicated three times. During the course of TE, one *in situ* reactor was improperly sealed (Williston, moderate moisture level), and the yield of this reactor was detected as an outlier.

The crude biodiesel yield was defined by the following formula:

$$\text{Yield} = \frac{\text{Crude biodiesel (g)}}{\text{Oil equivalent (g)}} \times 100\%$$

Impact of preparation method on biodiesel properties

Properties of biodiesel, including kinematic viscosity, oxidative stability, free fatty acids, moisture, and total glycerin content, were compared across both locations and 4 TE methods (3 iTE treatments and 1 CTE). The experiment was a 2 × 4 full factorial design replicated three times.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA). Tukey's Studentized Range (HSD) tests were performed for multiple comparisons ($p < 0.05$) using Minitab version 15 (Minitab Inc., State College, PA) software.

Safety Emphasis

Methanol, which was used in the transesterification procedure, is toxic. The minimum precautions when handling methanol include: 1. Eye-protection at all times; 2. Use of a well-ventilated work space (or a fume hood); 3. No one should work alone in the lab.

RESULTS AND DISCUSSION

Canola Seed Properties

The oil content and MC (wet basis) of the canola seeds grown at two ND locations in 2007 are shown in Table 7. Oil contents of seeds for Williston and Prosper samples were determined to be 37.8 and 44.8% on a dry basis, respectively. The samples from two locations were conditioned to three MC treatments; however, the resulting MCs were slightly different from the objective MCs.

Table 7. Oil and moisture contents of ground canola seed samples on a wet basis.

Location	Moisture Level	Oil (%)	Moisture (%)
Williston	**L**	37.8	0.0
	M	36.3	4.1
	H	35.4	6.7
Prosper	**L**	44.8	0.4
	M	42.9	4.6
	H	41.8	6.6

Precision of oil content measurement via about 20 g sample using the DA 7200 NIR is not available, but the precision is comparable to, although a little less than, measurement using the standard 300 g cup for breeders.

Table 8 presents five principal fatty acid compositions of the canola seed samples, which account for 91.7% and 87.5% of total fatty acids for Williston and Prosper seed samples, respectively. Saturated fatty acids, mainly the sum of palmitic (C16:0) and stearic (C18:0), account for 8% in the Williston samples. Its counterpart in Prosper samples is 6.5%. There are no remarkable differences in fatty acid profile between these two seed sources. The saturated fatty acid content of canola oil is typically 8%, which is much less than soybean oil (15%). This low saturated fatty acid content results in biodiesel with a desirable, lower cloud point, and is favored for use in cold conditions. The oleic acid of

canola biodiesel, a monounsaturated fatty acid, accounts for 53 to 59%. This high content of oleic acid creates canola biodiesel with both acceptable cold weather performance and oxidative stability (Knothe, 2005b).

Table 8. Fatty acid composition of canola seeds from two North Dakota locations in 2007.

	Content (wt. % of total fatty acid)	
Fatty acids	**Williston, ND**	**Prosper, ND**
Palmitic (C16:0)	4.9	4.3
Stearic (C18:0)	3.1	2.2
Oleic (18:1)	58.7	53.2
Linoleic (18:2)	18.0	20.1
Linolenic (18:3)	7.0	7.7

Effect of Seed MC on iTE Yield

The presence of moisture in the TE reaction is thought to markedly impede the reaction (Meher et al., 2006). In order to save operator time and achieve satisfactory conversion, it is important to determine if the seed moisture has negative effects on the iTE process given a realistic range of seed MC. Crude biodiesel, not yet subjected to water wash, was used for calculating yield. This is because the water wash may cause loss of biodiesel in certain samples and therefore introduced significant experiment variability.

Figure 10 shows biodiesel yields of samples treated with 3 MCs and 2 locations. All reactions were run at the same ratio of 350:1:1.17 of methanol/oil/KOH as well as the same operation conditions. One-way ANOVA shows seed MC ($p < 0.001$) significantly impacted iTE biodiesel yield. Reactions of low MC treatment (< 0.5%) yielded the most amount of crude biodiesel for both locations. The yield of the Williston biodiesel for the low MC treatment was 86.7%, and decreased about 30% when MC was increased to the high MC treatment (6.7%). Biodiesel yield of Prosper samples was also decreased with the

increasing MC, although was less affected compared with Williston samples. Drying canola seed to MC < 0.5% may extend operator time and increase production costs, but this step is recommended to achieve more than 80% yield.

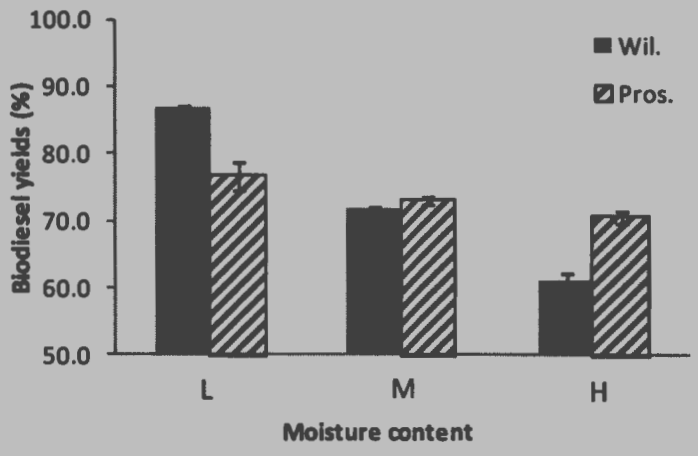

Figure 10. Impact of canola seed moisture content on crude biodiesel yield of the *in situ* transesterification. L: low seed moisture treatment; M: moderate seed moisture treatment; and H: high seed moisture treatment. Error bars represent one standard deviation (n=2 for the Wil. M sample; n=3 for other samples).

The effect of seed moisture shown in Figure 10 is similar to previous studies on iTE. Harrington and D'Arcy-Evans (1985b) found that biodiesel yield was slightly increased (about 5%) when sunflower was solvent-dried from the original 5.2% MC. Haas and Scott (2007) compared iTE yields of dry soy flakes and of 7.4% moisture, and found a 7% improvement after moisture removal. With comparable high yield, the moisture-removed samples resulted in 60% and 56% reduction in methanol and NaOH use, respectively (Haas and Scott, 2007).

Characteristics of iTE and CTE Biodiesel

For commercial use in the US, biodiesel needs to meet the ASTM D 6751 specifications for kinematic viscosity, oxidative stability, cloud point, cold soak filterability, and free fatty acid, moisture, total glycerin content and other quality traits. Some of the properties depend on the feedstock, which is impacted by genotype and growing environment. As a result, canola breeding programs need the above-mentioned biodiesel properties to be evaluated in order to make sure all breeding lines resulting in acceptable biodiesel quality. Experimental data of biodiesel properties are tabulated in Appendix A, Tables 12 to 19.

Figure 11 presents properties of all treatments in this study. Biodiesel moisture content ranged from 180 to 564 ppm, with most iTE samples slightly above the ASTM limit of 500 ppm and both TCE samples less than 300 ppm. Biodiesel samples made via three iTE methods were refined with anhydrous magnesium sulfate, while CTE samples were dried by vacuum heating. Thus, the difference in the refined biodiesel moisture content was likely due to the use of different drying methods. Total glycerin ranged from 0.018% to 0.246%. All samples meet the ASTM limit of 0.24%, except the Prosper CTE biodiesel. Use of the iTE method resulted in dramatic reduction in total glycerin, which may be due to the prolonged TE reaction time and to the introduction of a centrifugation step between water washes. The higher methanol:oil ratio would also shift equilibrium in favor of biodiesel formation. Kinematic viscosity, ranging from 4.69 to 4.90 cSt and all within the ASTM limit of between 1.9 and 6.0 cSt, was not impacted by either biodiesel preparation method or location. FFA content of most samples was slightly higher than the ASTM limit of 0.25%, and it was the only property significantly impacted by the growing location.

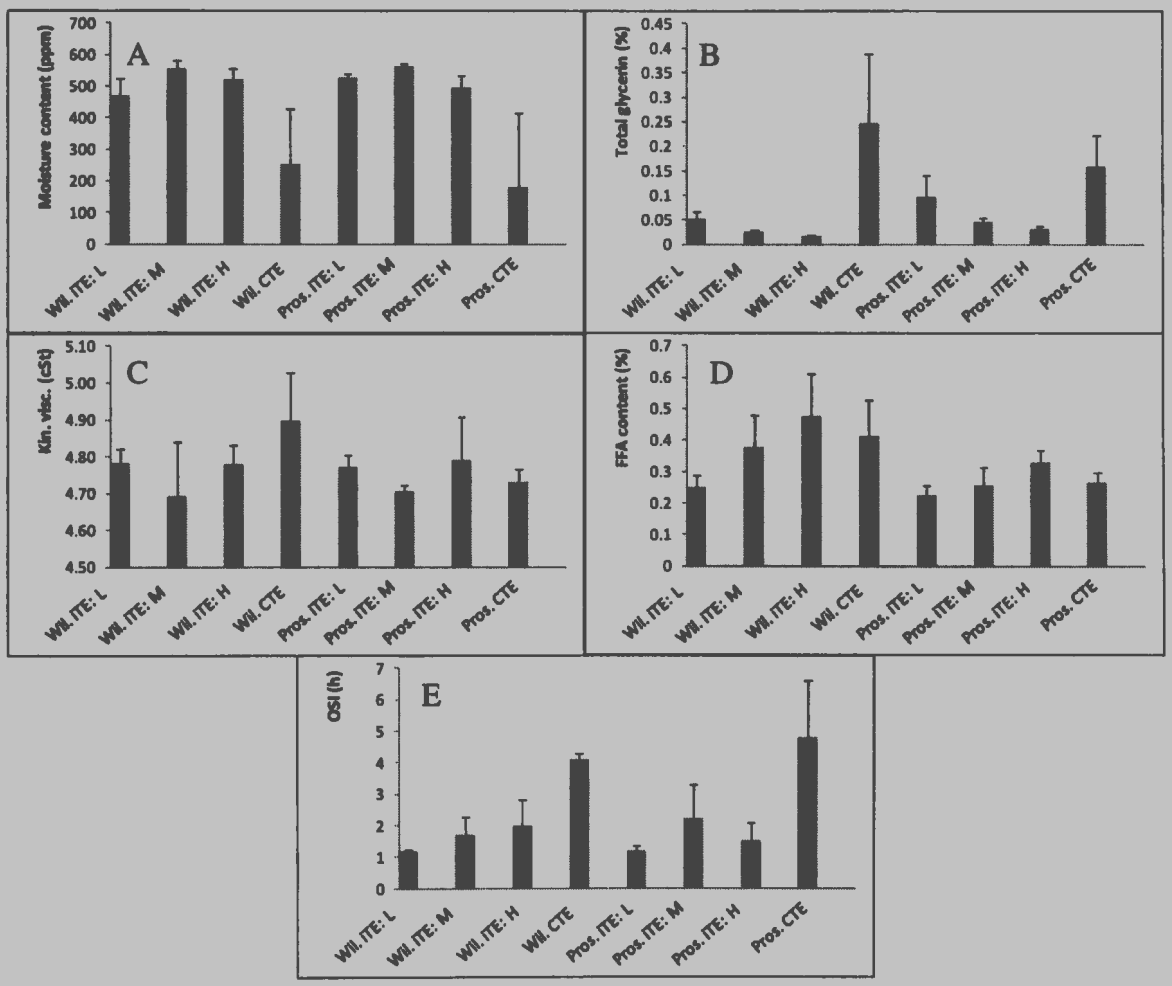

Figure 11. Characterization of canola biodiesel prepared via four different TE methods (3 iTE and 1 CTE methods). A, moisture content; B, total glycerin; C, free fatty acids content; D, kinematic viscosity; and E, OSI. Horizontal axis titles present different treatments. Error bar indicates one standard deviation from the mean (n=3).

OSI is a biodiesel property associated with feedstock oil qualities such as the fatty acid profile and the presence of antioxidants. A longer OSI indicates a longer biodiesel shelf life, and such biodiesel should show little decomposition during commercial storage. ASTM requires that the OSI be at least 3 h. This study obtained an average iTE OSI time of 2.4 h (σ = 1.0 h) across all iTE samples, and an average OSI time of 4.3 h (σ = 1.6 h) for the CTE samples. Moser (2008) reported an OSI time of 6.4 h for the conventional canola

biodiesel, which value was comparable to CTE biodiesel in our study, but much longer than that of iTE biodiesel in our study.

Figure 12 displays the conductivity curves against heating time during the OSI test at 110°C. The curve of the conventional biodiesel sample shows a reasonably long onset time, and is smoother than that of the iTE sample. The lack of onset time indicates a possibility that the biodiesel had already been stressed and natural antioxidants were consumed prior to the OSI test. A highly likely explanation is that the drying step had exposed canola flour with very high surface areas to air in a gravity convection oven at 70°C for overnight. Later studies in our group reduced the seed drying time to 3 h, and the resulting OSI was 8.0 h, longer than the corresponding conventional sample of 4.5 h (Haagenson et al., 2010). Similarly, Haas and Scott (2007) reported a 76% higher tocopherol level for biodiesel made through iTE than CTE, and hypothesized an OSI increase due to the higher tocopherol level in iTE biodiesel.

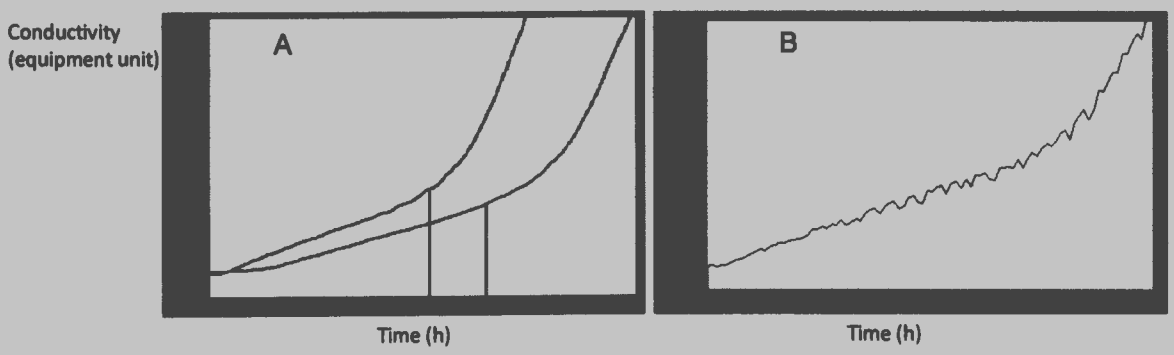

Figure 12. Time course curves of biodiesel during biodiesel OSI tests. The y axis indicates conductivity of ionized water by biodiesel volatile decomposition (oxidation) products, and x axis is the time period (h). A, well-shaped curves of CTE biodiesel samples, OSI = 3.1 and 3.9 h; B, curve of a typical iTE sample, OSI = 3.65 h.

In order to quantify contributions of the TE method and growing location, two-way ANOVA was performed on the evaluated biodiesel properties (Table 9). For biodiesel

moisture content, total glycerin, and kinematic viscosity, results show the method factor contributed the most proportion of the variance. The method has significant effect on the biodiesel properties of moisture content, total glycerin, and FFA content ($p < 0.05$).

Table 9. Proportion of variance (total mean squares) attributed to location, method, and interaction of location and method.

	Variance component (%)		
Biodiesel properties	**Location**	**Method**	**Location × Method**
Moisture content	0.3	96.7*	3.0
Total glycerin	20.2	76.6*	3.2
FFA content	68.9*	26.6*	4.5
Kinematic viscosity	25.3	42.0	32.7

Results are expressed as percentage of total mean squares. Results with an asterisk are significant at $p < 0.05$.

Comparison of Time Requirements of iTE and CTE

The iTE shows good potential for preparing biodiesel samples with acceptable yield and quality. In order to further compare the time requirements of iTE and CTE for biodiesel sample preparation, the duration of each step for both biodiesel preparation methods (Table 10) was estimated on the basis of a throughput of 40 samples per wk. Estimates were based on two shaker water baths and two hot plates as reaction platforms for the iTE and CTE, respectively (Figure 13).

The total operator time (O_k) of iTE is 6.6 h, and is only 72% of the total CTE operator time (9.15 h). Since O_k is defined as the time with operators involved, the lower total O_k indicates a significant advantage in sample preparation. Both the total unsupervised time (U_k) and total T_k of iTE are much less than those of the CTE.

One challenge associated with the iTE is that the quantity of biodiesel obtained in each reactor is too limited to complete evaluation of all desired properties. For example, the

Table 10. Time period estimations to process 40 samples per wk using iTE and CTE.

Method	k	SU_k (h)	O_k - SU_k (h)	O_k (h)	U_k (h)	T_k (h)
	1	0.1	1	1.1	0	1.1
	2	0.2	0	0.2	24	24.2
	3	0.3	0	0.3	16	16.3
	4	0.2	1	1.2	0	1.2
iTE	**5**	0.1	0	0.1	24	24.1
	6	0.1	0.5	0.6	0	0.6
	7	0.5	2	2.5	1.2	3.7
	8	0.1	0.5	0.6	24	24.6
	Total	1.6	5	6.6	89.2	95.8
	1	0.2	1.6	1.8	168	169.8
	2	0.5	0.85	1.35	0	1.35
	3	0.2	1.1	1.3	0	1.3
	4	0.2	1	1.2	0	1.2
CTE	**5**	0.05	0.8	0.85	0.5	1.35
	6	0.05	0.7	0.75	0	0.75
	7	0.1	1.2	1.3	0.9	2.2
	8	0.1	0.5	0.6	0	0.6
	Total	1.4	7.75	9.15	169.4	178.6

The k values from 1 to 8 represent different steps for each method as shown in Figure 8 and 9. SU_k: setup time for each step; O_k: operator time for each step; U_k: unsupervised time for each step; and T_k: total processing time for each step.

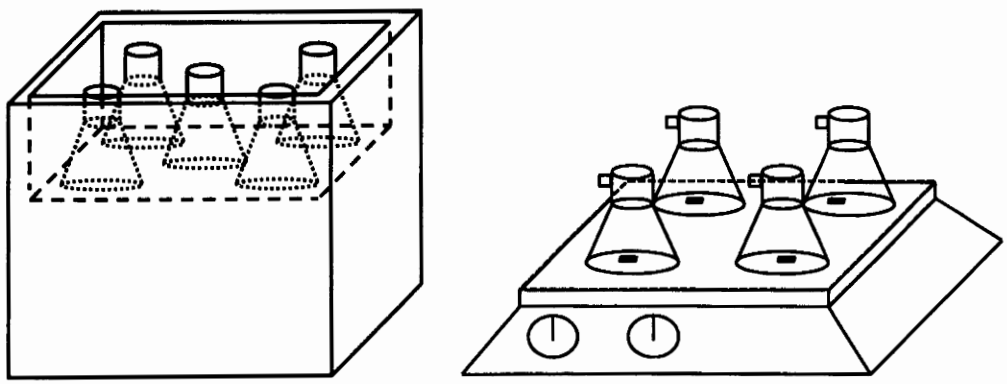

Figure 13. Thermostated/shaker water bath (left) and hot plate (right) as reaction platforms with full loads.

cold soak filtration test requires a 300 mL biodiesel sample for each measurement. This quantity is much beyond the capability of each iTE reactor (35 mL), and also beyond the seed amount that canola breeders could provide from experimental breeding lines. One possible solution is to scale down this filtration test to 25 mL to accommodate the iTE

method, in which is discussed further in Paper 2. The CTE requires much more seed (500 g) at the screw press step to set up the pressing and to prevent cross-contamination, while the minimum seed amount (100 g) for iTE depends of the biodiesel needed for quality evaluation. The amount required for CTE is significantly beyond what a seed breeder could provide, and shows an obvious disadvantage of CTE compared with iTE.

CONCLUSIONS

In this study, the iTE method resulted in acceptable biodiesel yields for all canola samples with moisture contents up to 6.7%. Properties (moisture content, total glycerin, and oxidative stability) of iTE biodiesel were comparable to or better than CTE biodiesel. In the follow-up study (Haagenson et al, 2010), an optimized seed drying step avoided the seed exposure to air at high temperature overnight, and improved the iTE OSI to 8 h. The iTE method reduces the processing time for each batch of 40 samples by 25% compared with the CTE method, given the equipment and operator availability of our lab. The iTE also reduces the seed amount required to prepare a biodiesel sample. Therefore, the iTE method shows significant advantages for use in canola breeding programs for biodiesel.

ACKNOWLEDGEMENTS

The authors are thankful for the research support from the North Dakota Agricultural Experiment Station and the North Dakota Center of Excellence for Oilseed Development. Research assistants Mukesh Kumar and Shay Richter are gratefully acknowledged for their technical support. Dr. Mukhlesur Rahman, canola breeder in the Department of Plant Sciences at North Dakota State University, is also acknowledged for providing canola seeds.

REFERENCES

ASTM. 2009. Standard specification for biodiesel fuel blend stock (B100) for distillate fuels, designation D 6751-09. West Conshohocken, Pa.: American Society for Testing and Materials.

Dunn, R.O. 2005. Cold weather properties and performance of biodiesel. In *The Biodiesel Handbook*, 83-121. G. Knothe, J. Krahl, and J. Van Gerpen, eds. 1st Ed. Champaign, Ill: AOCS Press.

Fukuda, H., A. Kondo, and H. Noda. 2001. Biodiesel fuel production by transesterification of oils. *J. Biosci. Bioeng.* 92(5): 405-416.

Georgogianni, K.G., A.G. Kontominas, P.J. Pomonis, D. Avlonitis, and V. Gergis. 2008. Alkaline conventional and in situ transesterification of cottonseed oil for the production of biodiesel. *Energy Fuels* 22(3): 2110-2115.

Haagenson, D.M., R.L. Brudvik, H. Lin, and D.P. Wiesenborn. 2010. Implementing an in situ alkaline transesterification method for canola biodiesel quality screening. *J. Am. Oil Chem. Soc.* 87(11): 1351-1358.

Haas, M.J., K.M. Scott, W.N. Marmer, and T.A. Foglia. 2004. In situ alkaline transesterification: An effective method for the production of fatty acid esters from vegetable oils. *J. Am. Oil Chem. Soc.* 81(1): 83-89.

Haas, M.J., and K.M. Scott. 2007. Moisture removal substantially improves the efficiency of in situ biodiesel production from soybean. *J. Am. Oil Chem. Soc.* 84(2): 197-204.

Harrington, K.J., and C. D'Arcy-Evans. 1985a. Transesterification in situ of sunflower seed oil. *Ind. Eng. Chem. Prod. Res. Dev.* 24(2): 314-318.

Harrington, K.J., and C. D'Arcy-Evans. 1985b. A comparison of conventional and in situ methods of transesterification of seed oil from a series of sunflower cultivars. *J. Am. Oil Chem. Soc.* 62(6): 1009-1013.

Knothe, G. 2005a. The history of vegetable oil-based diesel fuels. In *The Biodiesel Handbook*, 4-16. G. Knothe, J. Krahl, and J. Van Gerpen, eds. 1st Ed. Champaign, Ill: AOCS Press.

Knothe, G. 2005b. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Process. Technol.* 86(10): 1059-1070.

Knothe, G. 2008. 'Designer' biodiesel: optimizing fatty ester composition to improve fuel properties. *Energy Fuels* 22(2):1358-1364.

Meher, L.C., D.V. Sagar, and S.N. Naik. 2006. Technical aspects of biodiesel production by transesterification - a review. *Renewab. Sustain. Energy Rev.* 10(3): 248-268.

Moser, B.R. 2008. Influence of blending canola, palm, soybean, and sunflower oil methyl esters on fuel properties of biodiesel. *Energy Fuels* 22(6): 4301- 4306.

NDAWN. 2007. Fargo, N.D.: North Dakota State Climate Office. Available at: http://ndawn.ndsu.nodak.edu. Accessed 19 December 2009.

Qian, J.F., F. Wang, S. Liu, and Z. Yun. 2008. In situ alkaline transesterification of cottonseed oil for production of biodiesel and nontoxic cottonseed meal. *Biores. Technol.* 99(18): 9009-9012.

Ratnayake, W.M.N., and J.K. Daun. 2004. Chemical composition of canola and rapeseed oils. In *Rapeseed and Canola Oil: Production, Processing, Properties and Uses*, 38-78. F.D. Gunstone, ed. Boca Raton, Fla: CRC Press.

Siler-Marinkovic, S., and A. Tomasevic. 1998. Transesterification of sunflower oil in situ. *Fuel* 77(12): 1289-1391.

Tostenson, K., D. Wiesenborn, D. Haagenson, V. Hofman, K. McKay, B. Jenks, and S. Halley. 2007. Oil and biodiesel from canola having a high content of green seed. ASABE Paper No. RRV-07110. Fargo, N.D.: ASABE/CSBE North-Central Intersectional Conference.

USDA. 2008. North Dakota Canola Production Statistics: 2008. National Agricultural Statistics Database. Washington, D.C.: USDA National Agriculture Statistics Service. Available at: http://www.nass.usda.gov. Accessed 29 April 2009.

USDA-GIPSA. 2004. Grain Inspection Handbook-Book II Chapter 3: Canola, 13-15. Washington DC: Grain Inspection. Packers and Stockyards Administration.

Vargas-Lopez, J.M., D. Wiesenborn, K. Tostenson, and L. Cihacek, 1999. Processing of crambe for oil and isolation of erucic acid. *J. Am. Oil Chem. Soc.* 76(7):801–809.

PAPER 2: EFFECTS OF TRACE CONTAMINANTS ON COLD SOAK FILTERABILITY OF CANOLA BIODIESEL

Paper 2 was an edited and revised version of a paper presented at the ASABE Annual International Meeting in Pittsburgh, PA, June 20 – 23, 2010. Authors: Hongjian Lin, Darrin M. Haagenson, Dennis P. Wiesenborn, Scott W. Pryor, and Rachel Brudvik. Title: Effect of Trace Contaminants on Cold Soak Filterability of Canola Biodiesel. Paper number: 1009049. A revised version was submitted to *Fuel* as a manuscript on July 9, 2010. Hongjian Lin, the author of this thesis, is the first author of Paper 2. He designed and conducted the experiment in this paper and also wrote Paper 2, which was edited by the other co-authors.

ABSTRACT

A cold soak filtration test (CSFT; ASTM D 7501-09b) was included in B100 specifications under ASTM D 6751-09, bringing new challenges to biodiesel producers and researchers investigating B100 quality. For a plant breeding program evaluating canola biodiesel quality traits, rapid assessment of biodiesel quality is important. Typically, a limited amount of seed from new canola lines is available; therefore, obtaining the required volume of biodiesel for evaluating cold soak filterability (300 mL) is not possible. In order to rapidly screen canola breeding lines for B100 quality, cold soak filterability must be assessed with reduced volumes of biodiesel. The primary objective of this study was to evaluate the impact of saturated monoglycerides (SMG), glycerin, and soap on cold soak filterability. Biodiesel filtration time rapidly escalated when the SMG concentration was above 0.28%. The influence of SMG (0.04% to 0.46% w/w) on biodiesel precipitate formation was also evaluated. A regression model was generated to predict the filterability of biodiesel against the concentrations of these trace contaminants. The results will be instrumental to scaling down biodiesel CSFT for a canola breeding program.

INTRODUCTION

Biodiesel (fatty acid methyl esters or FAME) is a category of renewable fuels derived from triglycerides (TG) by transesterification (Van Gerpen and Knothe, 2005). It is considered an alternative to diesel for use in diesel engines. Despite its renewability, biodegradability and exhaust emission reduction, biodiesel has shortcomings in terms of cold weather performance (CWP) compared to diesel fuels (Krishna et al., 2007). When operated under winter conditions in Northern Plains of USA, biodiesel may impede engine performance through fuel line and fuel filter plugging (Dunn, 2009). Some quality standards have consequently been proposed for characterizing the CWP of biodiesel, and great effort has been taken to evaluate biodiesel CWP.

Compounds in biodiesel may have various melting points, and the higher melting point components may precipitate out of the liquid phase under winter conditions. Moser (2009) and Dunn (2005) summarized impacts of various components on the CWP of biodiesel based on the following tests: kinematic viscosity, cloud point, pour point, or cold filter plugging point. They concluded that the reduced CWP was primarily a result of high melting point (>25°C) compounds. Sterol glucosides in soy biodiesel (Bondioli et al., 2008; Pfalzgraf et al., 2007) and monoglycerides due to incomplete conversion (Van Gerpen et al., 1996; Selvidge, 2008) were also frequently reported to impair CWP. Blending biodiesel with diesel fuels improved CWP according to a series of response data of cloud point and pour point (Joshi and Pegg, 2007; Bhale et al., 2009).

Besides the above-mentioned quality tests, the American Society for Testing and Materials (ASTM) approved the cold soak filtration test (CSFT) ASTM D 7501-09b (ASTM, 2009) for determining the fuel filter blocking potential of biodiesel. This method

has been included in the most recent ASTM D 6751 specifications (2009) as the "cold soak filterability", with a maximum limit of 360 s for blending up to B20 (for use above -12°C).

The feedstock oil impacts the cold soak filterability of biodiesel (Sanford et al., 2009). Consequently, plant genetic or environmental factors influence the filterability of B100 by altering the fatty acid profile or minor constituent accumulation in oilseed lipids. It is therefore important, in canola breeding programs for biodiesel use, to evaluate the cold soak filterability of biodiesel from the different canola lines across multiple production areas. Due to seed increase limitations, canola breeders have limited seed supplies of advanced breeding lines, not allowing a CSFT to be routinely performed at the 300 mL volume of biodiesel required for one test. As a result, seed breeding programs will need a modified CSFT.

Objectives

Establishing a downscaled CSFT procedure would help assess filterability of canola B100; well-defined biodiesel standards representing a range of filterability would be useful for calibration. The primary objective of this study was to measure the impact of saturated monoglycerides (SMG), glycerin, and soap on the cold soak filtration time. Influence of SMG (with concentration from 0.04% to 0.46% w/w) was evaluated in terms of precipitate formation in canola biodiesel. A related objective was to determine the effect of refining (water wash) on biodiesel filterability and other biodiesel properties.

MATERIALS AND METHODS

Materials

Commercial edible-grade canola oil (Pure Wesson®, ConAgra Foods Inc., Omaha, NE) was bought locally, sealed and stored in the dark at room temperature. Potassium hydroxide and methanol of ACS reagent grade were both purchased from EMD Chemicals Inc., Gibbstown, NJ. A sample of SMG (Alphadim 90 SBK) prepared from soy oil with hydrogenation was kindly provided by Caravan Ingredients (Lenexa, KS). Glycerin was obtained from Mallinckrodt Baker Inc. (Phillipsburg, NJ). Four soap samples (sodium stearate, palmitate, linoleate, and oleate) were obtained from Nu-Chek Prep Inc. (Elysian, MN), and mixed to the proportions of typical canola fatty acid composition of 3%, 5%, 22%, and 70%, respectively. The resulting soap mixture was dissolved in methanol to prepare a 20 mg/mL soap solution.

Preparation of Biodiesel Samples

Alkaline transesterification

Canola oil (two 20 kg batches) was converted into biodiesel by alkaline transesterification in a steam-jacketed stainless steel tank reactor (30 D9MT, Lee Industries, Philipsburg, PA). The oil was pre-heated to 65°C at 50 kPa and stirred at 50 rpm. Vacuum and stirring were momentarily stopped for addition of the potassium hydroxide-methanol solution (0.16:1:6 molar ratio of catalyst:oil:methanol), and then resumed. The reaction proceeded for 1 h at 60°C and 80 kPa. The mixture was then allowed to settle for 3 h. The lower crude glycerin layer was drained from the tank reactor by gravity, and the upper crude biodiesel layer was heated at 65°C at 17 kPa for 30 min to remove the remaining methanol.

Crude biodiesel refining

The crude biodiesel was sequentially washed using 0.2 vol of 0.33% v/v acetic acid solution (50°C) and washed three times using 0.5 vol distilled water (50°C). After each wash 1.5 L of biodiesel was collected from the mixture, and dried for 15 min at 95°C and 17 kPa. The refined biodiesel was sealed and stored in a 20 L stainless steel container as the stock biodiesel until biodiesel quality analysis was performed.

Spiking biodiesel with trace contaminants

To evaluate the impact of trace contaminants on cold soak filterability, refined biodiesel was spiked with SMG, soap, and glycerin (see *Effects of SMG, Soap, and Glycerin on Filtration Time*). SMG pellets of calculated amounts were gravimetrically added to 300 mL biodiesel. Calculated levels of 20 mg/mL soap solution in methanol and glycerin were also added to 300 mL refined biodiesel via pipette. Dissolution of the contaminants into the biodiesel samples was aided by heating in a shaker water bath for 1 h at 60°C and 50 rpm, and then samples were heated at 17 kPa for 1 h at 60°C to evaporate the methanol introduced by the soap solution. Finally, samples were cooled to 25°C in a water bath for 1 h before initiating the CSFT.

Biodiesel Analysis

Fatty acid profile analysis

The fatty acid profiles of the feedstock canola oil, SMG, and biodiesel were quantified by gas chromatography (GC) according to the method described by Vick et al. (2004) and Espinoza-Perez et al. (2009) with minor modifications. Fatty acid concentrations were expressed as a weight percentage of the oil. Weight fraction of the

total saturated fatty acids (SFA) of canola oil, SFA% w/w, was calculated by combining the concentrations of palmitic and stearic acids.

Fourier transform infrared spectroscopy

Samples of canola biodiesel and sediment (formed at the bottom of a container of biodiesel after 1 mo storage) were analyzed by the Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) with 4 cm^{-1} resolution in the wavenumber range of 4000 to 650 cm^{-1}. Spectra were averaged over 32 scans. These spectra were used to determine the presence of functional groups in the samples, including hydroxide group, ester carbonyl, and carboxylate ion.

Total and free glycerin, and SMG concentration

Total glycerin was expressed as the sum of the weights of the bound glycerin in mono-, di-, and triglycerides, as well as free glycerin, relative to the weight of FAME. The total glycerin of canola biodiesel was quantified by the SafTest according to the manufacturer's recommendations (MP Biomedical, Solon, OH). Reagents in the SafTest cleaved molecules of bound glycerides (mono-, di-, and triglycerides). The resulting glycerin was digested enzymatically, and the break down product was measured spectrophotometrically at 570 nm. The free glycerin content of refined biodiesel was obtained by comparing the total glycerin reading with that of a sample washed 7 times.

The SMG concentration in refined biodiesel was estimated as follows:

$$[\mathrm{SMG}] = \frac{W_{SMG}}{W_{FAME}} 100\% = \left(\frac{W_G}{W_{FAME}}\right)\left(\frac{M_{Bound}}{M_G}\right)\left(\frac{M_{MG}}{M_{Bound}}\right)\left(\frac{MW_{MG}}{MW_G}\right)\left(\frac{W_{SMG}}{W_{MG}}\right)100\% \qquad (1)$$

where W_G, W_{FAME}, W_{SMG}, and W_{MG} are weights (g) of glycerin (in free and bound forms), FAME, SMG, and MG, respectively; M_{Bound}, M_G, and M_{MG} are amounts (mol) of bound glycerides, total glycerin, and monoglycerides, respectively; and MW_{MG} and MW_G are

molecular weights (g/mol) of monoglycerides and free glycerin, respectively. From the study of the effect of water wash on the total glycerin level, the mole fraction of bound glycerides in the total glycerin ($\frac{M_{Bound}}{M_G}$) was assumed to be 80% mol/mol. The mole fraction of MG in the bound glycerides ($\frac{M_{MG}}{M_{Bound}}$) was assumed to be 90% MG mol/mol. The weight fraction of SMG in the MG ($\frac{W_{SMG}}{W_{MG}}$) was assumed equal to the weight fraction of saturated fatty acids (5.9% SFA w/w) in canola biodiesel.

Soap content

Soap content was determined by titration in the presence of bromophenol blue indicator. The biodiesel was titrated with 0.01 N HCl solution until the color turned yellow as an indication of hydrochloric acid titration end point (pH=4.6) (Van Gerpen et al., 2004).

Kinematic viscosity, moisture content and acid number

Kinematic viscosity of biodiesel at 40°C was measured with Cannon-Fenske Capillary Viscometer Tubes (Routine 75, Technical Glass Products, Inc., Painesville Twp., OH) following ASTM D 445. Moisture content was determined with a Karl Fischer coulometric titrator DL32 (Mettler Toledo, Columbus, OH). Acid number was determined by KOH titration, and expressed as mg KOH/g biodiesel.

Cold soak filtration test

The cold soak filterability was measured according to the cold soak filtration test (ASTM D 7501-09b; CSFT). Biodiesel (300 mL) was stored at 4°C for 16 h, and then transferred to a water bath at 25°C for 2 h, or for 4 h if precipitate was present. The incubated biodiesel was then filtered through a 0.7 μm glass microfiber filter (Grade No. 151, Ahlstrom, Mt. Holly Springs, PA) under 16 to 31 kPa. The time required for the

biodiesel to pass through the filter was recorded as the filtration time (the unit of cold soak filterability).

A downscaled filtration test was based on a filtration system with an effective diameter of 16 mm. The volume of biodiesel used in each measurement was 25 mL. The same experimental procedure, temperature and vacuum were employed when obtaining the filtration time.

Quantification of Precipitates in Spiked Biodiesel

For the biodiesel samples spiked with SMG, precipitates (insoluble materials) were detected after cold incubation/filtration. The amount of precipitate was determined after CSFT, but the quantification method was dependent upon the initial concentration of SMG. For treatments containing ≤0.31% SMG, the post CSFT filter was rinsed with *n*-heptane, dried, and the mass increase was recorded. For biodiesel samples containing >0.31% SMG, additional refining steps were included, because a thick layer of sediment accumulated on the filter preventing complete filtration. For those samples, the sediment was transferred to a 50 mL centrifuge tube, and the supernatant was transferred to a filter flask assembly where filtration was completed with a fresh filter. The filter mass increase was reported as previously described. The sediment was washed with 20 mL *n*-heptane, vortexed, and centrifuged at 2100 × g for 15 min, and the washing steps were repeated three times to remove trace FAME. The remaining *n*-heptane was evaporated in a vent hood at room temperature, and the mass of the second filter and recovered sediments were combined.

Experimental Design

Impact of refining on filtration time and other qualities

Samples of crude biodiesel and biodiesel from each washing stage (see *Crude Biodiesel Refining*) were analyzed in duplicate for cold soak filterability, acid value, kinematic viscosity, total glycerin, soap, and moisture content. Data were analyzed using the one-way analysis of variance (ANOVA). Tukey's Studentized Range (HSD) tests were then performed for multiple comparisons using SAS 9.2 (SAS Institute, Cary, NC).

Impact of SMG on filtration time and precipitate formation

Different levels of SMG were added to biodiesel to evaluate its effects on the filterability and on the precipitate formation of biodiesel. The stock biodiesel had a low SMG level (0.04% w/w). The CSFT was conducted across 5 levels of SMG (0.04%, 0.16%, 0.21%, 0.26%, and 0.31% w/w) replicated three times, while mass of precipitates was quantified across 8 levels (0.04%, 0.16%, 0.21%, 0.26%, 0.31%, 0.36%, 0.41%, and 0.46% SMG w/w) relative to the refined biodiesel sample.

Effects of SMG, soap, and glycerin on filtration time

Evaluation of impact of trace contaminants (SMG, soap, and glycerin) on filterability of canola biodiesel was conducted with a 2^3 factorial central composite design (CCD) (Myers and Montgomery, 1995). The value of α, the axial distance, was chosen to be 1 to keep the investigated range within the region of operability. The low and high levels of trace contaminant additions were: 0.04 and 0.16% for SMG (X_1), 0.04 and 0.30% for glycerin (X_2), and 0 and 0.02% for soap (X_3). The detailed experimental design is presented in Appendix B. Response surface methodology (RSM) was used to analyze the experimental results. Filtration time (t), the response variable, was evaluated as a function of concentrations of the three independent variables (assuming no three-way interactions):

$$t = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j=2}^{3} \beta_{ij} X_i X_j + \varepsilon \quad (2)$$

where βs are the regression coefficients, and ε is a statistical error, a term representing sources of variability not included in the response function.

Experiment results were evaluated with ANOVA at a 95% level of confidence in order to determine the significance of the effect of the linear, quadratic, and interactive terms of the three variables. This analysis included the Fisher's *F*-test, Student's *t*-value, the associated probabilities *p(F)* and *p(t)*, and the coefficient of determination R^2. Before regression analysis, outliers were detected by checking the normality and studentized residuals. Minitab version 15 (Minitab Inc., State College, PA) software was used for statistical analysis.

RESULTS AND DISCUSSION

Composition of Canola Biodiesel and SMG Pellets

Oleic, linoleic, and linolenic acids are the three principal unsaturated fatty acids (UFA), and totaled 92.9% of canola biodiesel. The monounsaturated methyl ester, methyl oleate (C18:1), was the predominant component. The SMG pellets had a high level of SFA (98.3%). The main components of the pellets were monopalmitin (11.9%) and monostearin (85.6%) which have high melting points of 77°C and 82°C, respectively.

Cloud point is an important parameter for determining the cold weather performance of biodiesel (Dunn, 2009). When bound glycerides are present in biodiesel, e.g. at concentration of 0.1 wt % of SMG, cloud point increases (Dunn, 2005). In the absence of SMG, cloud point largely depends on the amount of high melting point esters, such as methyl palmitate (C16:0, 30°C melting point) and methyl stearate (C18:0, 39°C melting point), regardless of the levels of low melting point unsaturated esters (Knothe, 2008). The two principal saturated fatty acids of canola oil, palmitic and stearic acids, accounted for 5.9 wt %; canola biodiesel generally has a better cloud point than soy biodiesel because of its lower level of total SFA (Knothe, 2008; Canakci and Sanli, 2008).

Effects of Biodiesel Refining on Filterability and Related Qualities

The composition of crude biodiesel is complex, and refining is required to meet ASTM standards. The water wash refining method is widely applied in industrial biodiesel production, and was chosen for this study, because it works better than the dry wash method based on ion exchange resins or magnesium silicate, especially in terms of glycerin removal (Berrios and Skelton; 2008).

The impact of multiple wash steps on biodiesel quality is presented in Table 11. The total glycerin was significantly reduced by water wash from 0.449% to 0.259%, while the further reduction was not significant after the second wash. This can be explained by the fact that the free glycerin is easily dissolved in wash water while bound glycerides are not. After free glycerin was removed, the level of total glycerin in biodiesel was stable, and was derived from the glycerin part of bound glycerides. In contrast, the soap content consistently decreased with each of the four washes from 1322 ppm to 118 ppm, due to the solubility of potassium soap in warm water and the equilibrium between soap and free fatty acids discussed below.

Table 11. Characterization of crude canola biodiesel (0 × water wash) and biodiesel washed up to four times.

Water wash	Total glycerin		Soap		Filterability		Acid number		Kin. Viscosity	
	%		ppm		s		mg KOH/g		cSt	
0 ×	0.449	a	1322	a	104	a	0.068	b	4.65	b
1 ×	0.369	b	1016	ab	67.5	b	0.098	ab	4.8	a
2 ×	0.295	c	563	abc	60	b	0.151	ab	4.7	ab
3 ×	0.263	c	288	bc	56	b	0.161	a	4.6	b
4 ×	0.259	c	118	c	62	b	0.142	ab	4.7	ab
ASTM limit	<0.24		NA		<360		<0.50		1.9 to 6.0	

Letters following the means (n=2) indicate Tukey grouping, and means with different letters are significantly different ($p < 0.05$).

The cold soak filtration time of crude biodiesel was 104 s, and was significantly higher than biodiesel washed 1 or more times (from 67.5 to 56 s). However, even the crude sample was well within the ASTM limit of 360 s, despite high levels of total glycerin and soap. This distribution of filtration time is very different from our previous observations in which some samples had much larger filtration times (> 700 s). This difference could be due to the thermal history of these samples, e.g., the difference in storage time before conducting CSFT.

The insoluble materials in the crude biodiesel formed a stable suspension with only minor phase separation during 1 mo storage. The Tyndall effect, a special instance of diffraction, was observed in crude biodiesel samples; the crude biodiesel acts as a colloid in which trace contaminants form dispersed particles and compromise filterability. For washed samples, the Tyndall effect was not observed, but dark sediment was observed at the container bottom after 1 mo storage.

The dark sediment mentioned above was characterized by FT-IR analysis (Appendix C). The characteristic ester carbonyl stretching peak at 1742 cm^{-1} observed in biodiesel is much less pronounced in the sediment, suggesting limited amounts of FAME or bound glycerides. Two regions identified in the spectrum of dark sediment show characteristic peaks at 3276 cm^{-1}(hydroxide absorption) and 1561 cm^{-1} (carboxylate ion), which indicate the presence of glycerin and soap, respectively (Mirghani et al., 2002).

The acid number of crude biodiesel was lower than that of refined biodiesel, but all samples were within the ASTM limit of 0.5 mg KOH/g. When preparing the catalyst of potassium methoxide, water was introduced to the mixture as a by-product of the reaction between methanol and KOH. The resulting water helped hydrolyze FAME into free fatty acids (Drapcho et al., 2008), and then the resulting free fatty acids reacted with KOH to form potassium soap:

$$RCOOCH_3 + H_2O \leftrightarrow RCOOH + CH_3OH \quad (3)$$

$$RCOOH + KOH \leftrightarrow RCOOK + H_2O \quad (4)$$

When biodiesel was subjected to water wash, the equilibrium between soap and free fatty acids was shifted to favor conversion of potassium soap back to free fatty acids. This shift of equilibrium explains the increase of the acid number in the water washed biodiesel.

Due to the high level of moisture present in biodiesel after the water wash, moisture was removed by vacuum drying at 95°C to meet the ASTM D 6751 limit of 500 ppm for moisture content. As a result, moisture levels of all samples except the 3 × wash (529 ppm) were within the limit. The one exception was due to accidental backflow of condensate to the biodiesel during vacuum drying.

Kinematic viscosity of all biodiesel samples was within the ASTM limit, and showed no trend with respect to the number of washes. These data were comparable to kinematic viscosity of canola biodiesel in the literature, e.g., 4.42 cSt (Moser, 2008), 4.53 cSt (Knothe, 2008), and 4.6 cSt (Albuquerque et al., 2009).

Impact of SMG Spiked in Canola Biodiesel

Due to incomplete conversion, some bound glycerides remain in biodiesel. The average total glycerin of the refined canola biodiesel was 0.24%. The SMG concentration of the canola biodiesel was estimated from Eq. (1) to be 0.04%.

Purchased SMG prepared from soybean was added to canola biodiesel to evaluate the impact of SMG on filtration time and precipitate formation (Figure 14). Biodiesel filtration time slightly increased when the SMG concentration was increased from 0.04% to 0.21% (Figure 14 A). Filtration time still passed the ASTM D 6751 limit of 360 s when biodiesel was spiked to 0.26% SMG. Increasing the amount of SMG above 0.26% resulted in a filtration time that was more sensitive to SMG content, was less repeatable, and failed to meet the ASTM limit. For biodiesel samples with 0.31% SMG, filtration time was too long (> 2 h) to be precisely evaluated, and had very poor repeatability (σ = 2051 s). Samples with SMG concentration higher than 0.31% were not subjected to the CSFT due to the filtration time being beyond the useful range of this test.

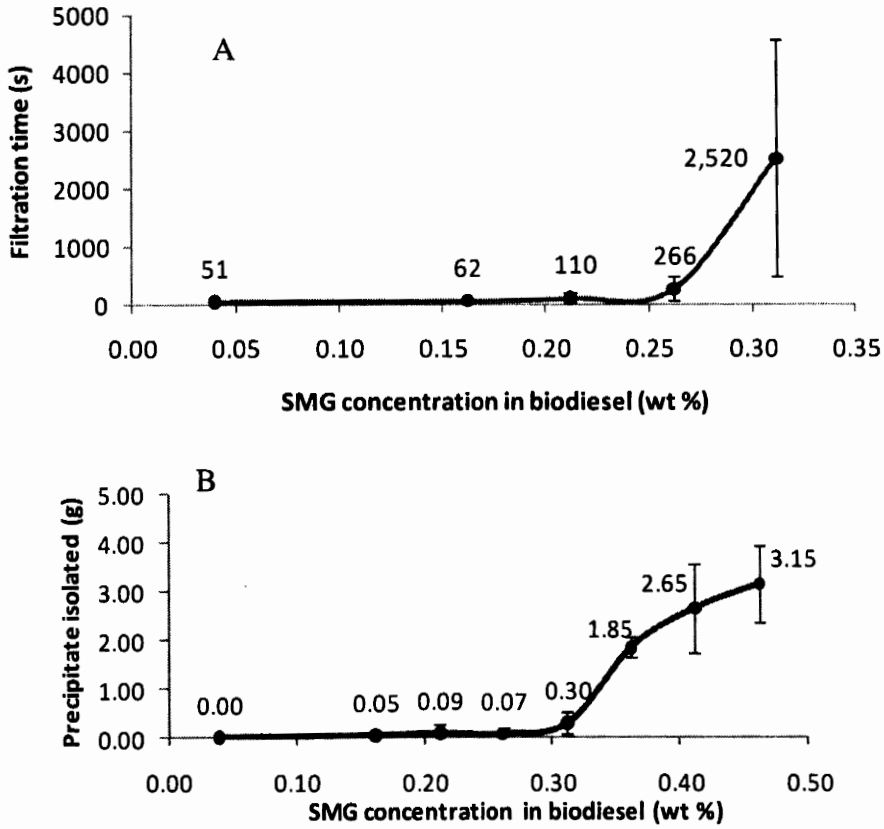

Figure 14. Cold soak filtration characteristics of canola biodiesel samples with different SMG concentrations. A: Filtration time of canola biodiesel samples with different SMG concentrations in refined biodiesel; B: Mass of precipitate isolated from 300 mL canola biodiesel samples with different SMG concentrations in refined biodiesel. Error bars suggest standard deviations of the mean (σ).

A similar effect of MG on filterability was found by Selvidge (2008) who tested the filterability of soybean biodiesel with different total glycerin and MG contents using the draft National Biodiesel Board filtration time test; this test uses a 1.6 μm filter rather than a 0.7 μm filter as specified in ASTM D 7501-09b. Filtration results demonstrated that MG addition increased filtration time, especially when the amount of MG was higher than 0.45%. However, this research did not point out the relationship between filtration time and specific SMG concentration. The author also analyzed flocculent solids isolated after

biodiesel refrigerated storage. The SMG (monopalmitin and monostearin) concentrations were 100 times more than their concentrations in the biodiesel liquid phase, and therefore monopalmitin and monostearin were concluded to be the major problematic impurities compromising biodiesel filterability.

Pfalzgraf et al. (2007) examined the influence of MG (0 to 1.0%) on the filterability of distilled soybean biodiesel by the modified ASTM D 6217 method. The authors mentioned that MG components, especially SMG, were viewed to affect filterability of soy biodiesel due to crystallization, although their impact was not as dramatic as that of sterol glucosides or soap. However, their maximum level of MG corresponded to 0.15% SMG, and they did not distinguish between the effect of SMG and unsaturated MG. For a sample with 10,000 ppm MG (or 0.15% SMG), 40 ppm soap, and 500 ppm water, the filtration time was 106 s (σ = 1 s), well within the ASTM limit. Comparing their data with this study, it is clear that SMG plays a more important role in compromising biodiesel filterability than unsaturated MG. Our unpublished studies also showed spiking biodiesel with unsaturated MG up to 1.1% had little effect on filtration time.

The filtration time, t, can be modeled by the cake filtration equation (Cheremisinoff, 1998):

$$t = \frac{r \mu x}{2 \Delta p A^2} V_b^2 + \frac{R_f \mu}{\Delta p A} V_b \qquad (5)$$

where r is specific volumetric resistance of cake; μ is the dynamic viscosity of the filtrate; x is the ratio of precipitate to the whole liquid volume; Δp is the pressure difference across the cake/filter; A is the effective surface area of glass fiber filter; V_b is biodiesel volume; and R_f is the resistance from the filter paper. All parameters in Eq. (5) are held constant during the CSFT, except r, μ and x. These parameters may be impacted by the amounts of

trace contaminants, which in turn depend on the feedstock oil, degree of oil conversion, biodiesel refining and long term storage. Of these three parameters, a change in x most likely accounts for the abrupt change in filtration time shown in Figure 14 A; therefore, the amount of precipitate in the SMG-spiked biodiesel was quantified in this study.

The biodiesel samples with up to 0.21% SMG concentration were translucent, which suggested that most SMG remained dissolved at such concentrations. The quantity of recovered precipitate was not influenced by SMG concentration at concentrations up to 26%. The mass of precipitate was 14%, 20%, and 12% of the SMG addition for the 0.16%, 0.21%, and 0.26% SMG treatment samples, respectively (Figure 14 B). These ratios show that part of the SMG spiked with canola biodiesel remained soluble in it.

For the sample with 0.31% SMG, the precipitate recovery rate increased slightly to 41% of the SMG addition; however, the filtration time rose dramatically to 2520 s. This small increase in precipitates corresponded to a sudden increase of filtration time indicated that SMG began to crystallize and compromise biodiesel filterability at around 0.31% SMG content. Although a dramatic effect of filtration time at 0.31% SMG was suggested by Eq. (5) due to the precipitate increase, this shift was much greater than that predicted. For the three heavily spiked (0.36%, 0.41%, and 0.46% SMG) samples, the total glycerin was evaluated for the liquid phase after 2 wk storage at 25°C. The average total glycerin was 0.28% (σ = 0.001%); from this, the SMG concentration was estimated to be 0.28%, and is indicative of SMG solubility in canola biodiesel.

The weight of isolated precipitate was 1.2, 1.7, and 1.8-fold higher than the SMG addition for the 0.36%, 0.41%, and 0.46% SMG treatments, respectively. The sudden shift of the weight of isolated precipitate from SMG concentration of 0.31% to 0.36% samples

may be partly attributed to the modified isolation method required for the heavily spiked samples beginning with 0.36% SMG (see *Quantification of Precipitates in Spiked Biodiesel*). The FAME was not easily removed from the precipitate with *n*-heptane rinses. FAME adhesion was confirmed by GC analysis of the CSFT filter cake, which showed the majority (74%) was oleic (C18:1) and linoleic (C18:2) acids from biodiesel itself.

Impact of SMG, Glycerin, and Soap on Biodiesel Filterability

The initial baseline concentrations of SMG (X_1), glycerin (X_2), and soap (X_3) in the stock biodiesel were estimated as 0.04%, 0.04%, and 0.00%, respectively. SMG, glycerin, and soap were further added to the refined biodiesel to evaluate their impact on canola biodiesel filterability. One of the duplicate samples in each of three treatments had unacceptably high studentized residuals; therefore, these three data points of the 34 total were deemed outliers (Sen and Srivastava, 1990), and removed prior to developing a regression model. The regression model would be used to predict biodiesel filterability from measured concentrations of the three trace contaminants.

Effects of X_2, X_3, X_3^2, X_1X_2, X_1X_3, and X_2X_3 were statistically significant (p-value < 0.05). The backward regression technique required keeping the term X_1 because of its significance in interaction terms, but eliminated statistically insignificant terms of X_1^2 and X_2^2. Based on these results, the regression model could predict the filtration time of canola biodiesel given SMG, glycerin, and soap concentrations ($-1 \leq X_1, X_2, X_3 \leq +1$) (Figure 18):

$$t = 61.5 - 3.2\,X_1 + 7.7\,X_2 + 14.6\,X_3 - 7.1\,X_1X_2 - 7.4\,X_1X_3 - 4.8\,X_2X_3 + 21.0\,X_3^2 \quad (6)$$

ANOVA results show that this model (p-value = 0.000) predicts the experiment results well with an R^2 of 87.9% and adequate goodness-of-fit test (lack-of-fit: p-value =

0.310). Residuals did not follow any particular pattern, and the normality assumption for regression analysis was found satisfactory by checking the normal probability plot.

Contour plots were generated from Eq. (6) to visualize effects of the three variables. When soap or glycerin concentration was held at the lowest level, increasing SMG had a minor negative impact on filtration time (Figure 15 A and B). This is not surprising as the maximum SMG treatment (+1, or 0.16%) did not increase filter precipitate recovery or impede filtration as noted in the previous section, mainly because this value was lower than the estimated SMG solubility (0.28%) in canola biodiesel at 25°C.

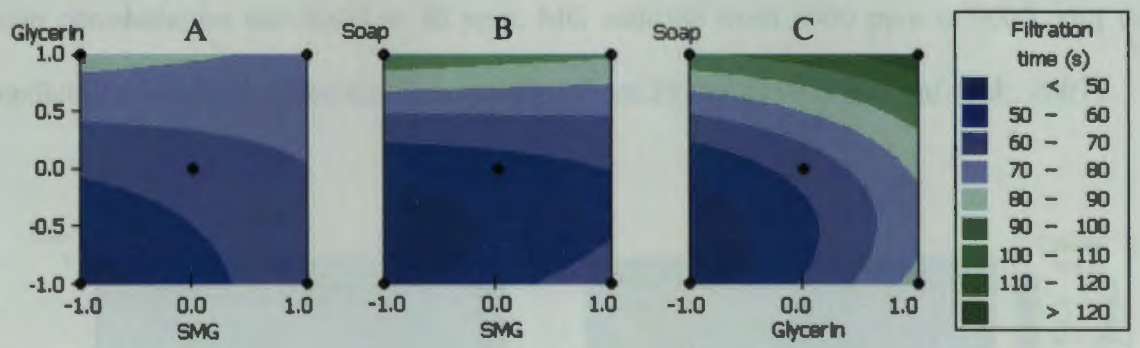

Figure 15. Contour plots of cold soak filtration time of canola biodiesel. A: the effect of glycerin and SMG; B: the effect of soap and SMG; and C: the effect of soap and glycerin. The third variable (soap, glycerin, and SMG, respectively) was held at the lowest level (-1) in generating these plots.

Low levels of contaminants were expected to result in biodiesel with acceptable filterability, and all predicted values in the sampling region were within the ASTM D 6751 limit of 360 s. When the SMG level was held at the lowest level, increasing glycerin and soap concentrations increased filtration time from less than 50 s to more than 110 s (Figure 15 C). Glycerin forms emulsions in biodiesel, which increases the dynamic viscosity and consequently the filtration time (see Eq. (5)). The addition of soap has a more dramatic

effect on filtration time compared with glycerin. Soap is insoluble in biodiesel, and could form translucent gels/films in biodiesel. It may also interact with glycerin to generate a colloid which impedes filtration. Similarly, Pfalzgraf et al. (2007) found that filterability of soy biodiesel was very sensitive to soap even around the concentration of 40 ppm.

At the highest soap or glycerin levels (+1 coded level, Figure 16), addition of SMG surprisingly improved biodiesel filterability. A possible explanation was that the dissolved SMG acted as a co-solvent for soap or glycerin due to its amphiphilic property, reducing the amount and size of solid particles or gels which clogged the glass fiber filter. A similar effect of MG on filterability was observed in a previous study by Pfalzgraf et al. When soap concentration was fixed at 30 ppm, MG addition from 3000 ppm to 9000 ppm was predicted to slightly reduce the filtration time from 237 to 221 s (Pfalzgraf et al., 2007).

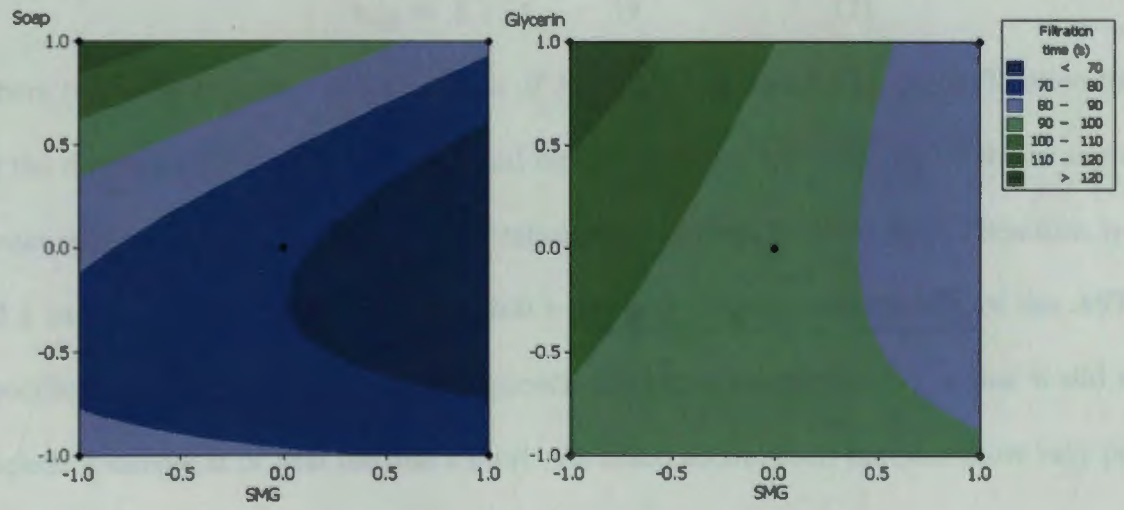

Figure 16. Contour plots of cold soak filtration time of canola biodiesel when the third variable (coded) of trace contaminants was held at level +1.

Downscaled Method for Cold Soak Filterability

Biodiesel samples which fail the visual inspection for sediment present will be likely to have poor cold soak filterability. In this study, biodiesel samples spiked with 0.21% SMG were evaluated for filterability. The samples were observed to contain sediment, and showed extraordinarily poor repeatability of filtration time (σ = 312 s; n = 12) although having a marginally acceptable average value of 362 s. In contrast, a well-refined biodiesel sample had a very good average value (51 s; n = 7) and very good repeatability (σ = 1.7 s). This result suggests that a downscaled model might accurately predict the filtration time for samples in the absence of visible gels or sediments.

A series of canola biodiesel samples (n = 15) possessing filtration times ranging from 54 to 326 s, which were randomly obtained from different batches of TE reactions (Appendix D), were used for the purpose of calibration. The prediction equation was estimated by least squares method as the follows:

$$t_{300} = 5.75\, t_{25} - 39 \qquad (7)$$

where t_{300} is the cold soak filtration time of 300 mL biodiesel, and t_{25} is the filtration time of the downscaled filtration system based on 25 mL biodiesel. This model shows a good linear relationship between these two filtration times with an R^2 of 99.26%. Therefore, t_{25} = 42 s and t_{25} = 69 s correspond to the 200 s and 360 s limits, respectively, of the ASTM specification. One limitation to the approach used to generate Eq. (7) is that it did not include a sample at or near the 360 s limit. As noted above, such samples show very poor repeatability.

CONCLUSIONS

The canola biodiesel prepared for this study had very good cold soak filterability; nevertheless, such performance should not be taken for granted in an oilseed breeding program. Assessing the cold soak filterability with a reduced volume of biodiesel, instead of the 300 mL required by ASTM D 7501-09b CSFT, is necessary for developing a high throughput screening method for canola breeding lines for B100. Biodiesel standards possessing a range of filtration time covering 360 s and good repeatability would be useful for the calibration of a downscaled method. For this purpose, biodiesel samples of varying purity were prepared through multiple water wash, but the filtration time range was limited from 62 s to 104 s. Spiking biodiesel with contaminants of interest in concentrations resembling those that might be encountered in practice was also evaluated; the filterability range was still limited from 50 s to 120 s. When the SMG concentration was increased above 0.16%, the filtration time was greatly increased, but repeatability was not satisfactory. Nevertheless, the results of these studies together with the cake filtration model provided important insights that will help in developing a downscaled CSFT method.

ACKNOWLEDGEMENTS

The authors are thankful for the research support from the North Dakota Agricultural Experiment Station and the North Dakota Center of Excellence for Oilseed Development. Former research specialist Rachel Brudvik, and research assistants Mukesh Kumar and Katie McKinnon (NDSU Agricultural and Biological Engineering Department) are acknowledged for their technical support. The GC analysis provided by Leonard Cook, (USDA-ARS, Fargo, ND) is gratefully acknowledged. The authors are also thankful to Enrico Sassi (NDSU English Department) for providing scientific writing consulting, to Curt Doetkott (NDSU Information Technology Services) for providing statistical consulting.

REFERENCES

Albuquerque, M.C.G., Y.L. Machado, A.E.B. Torres, D.C.S. Azevedo, C.L. Cavalcante Jr., L.R. Firmiano, and E.J.S. Parente Jr. 2009. Properties of biodiesel oils formulated using different biomass sources and their blends. *Renew. Energ.* 34(3): 857-859.

ASTM. 2009. Standard test method for determination of fuel filter blocking potential of biodiesel (B100) blend stock by cold soak filtration test (CSFT), designation D 7501-09b. West Conshohocken, Pa.: American Society for Testing and Materials.

Berrios, M., and R.L. Skelton. 2008. Comparison of purification methods for biodiesel. *Chem. Eng. Journal* 144(3): 459-465.

Bhale, P.V., N.V. Deshpande, and S.B. Thombre. 2009. Improving the low temperature properties of biodiesel fuel. *Renew. Energ.* 34(3): 794-800.

Bondioli, P., N. Cortesi, and C. Mariani. 2008. Identification and quantification of steryl glucosides in biodiesel. *Eur. J. Lipid Sci. Technol.* 110(2): 120-126.

Canakci, M., and H. Sanli. 2008. Biodiesel production from various feedstocks and their effects on the fuel properties. *J. Ind. Microbiol. Biot.* 35(5): 431-441.

Cheremisinoff, N.P. 1998. *Liquid Filtration*. Boston, MA: Butterworth-Heinemann.

Drapcho, C.M., N.P. Nhuan, and T.H. Walker. 2008. *Biofuels Engineering Process Technology*. New York, NY: McGraw-Hill,

Dunn, R.O. 2005. Cold weather properties and performance of biodiesel. In *The Biodiesel Handbook*, 83-121. G. Knothe, J. Krahl, and J. Van Gerpen, eds. 1st Ed. Champaign, Ill: AOCS Press.

Dunn, R.O. 2009. Effects of minor constituents on cold flow properties and performance of biodiesel. *Prog. Energy Comb. Sci.* 35(6): 481-489.

Espinoza Pérez, J.D., D.M. Haagenson, S.W. Pryor, C.A. Ulven, and D.P. Wiesenborn. 2009. Production and characterization of epoxidized canola oil. *Trans ASABE* 52(4): 1-9.

Joshi, R.M., and M.J. Pegg. 2007. Flow properties of biodiesel fuel blends at low temperatures. *Fuel* 86(1-2): 143-151.

Knothe, G. 2008. "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. *Energ. Fuels* 22(2): 1358-1364.

Krishna, C.R., K. Thomassen, C. Brown, T.A. Butcher, M. Anjom, and D. Mahajan. 2007. Cold flow behavior of biodiesels derived from biomass sources. *Ind. Eng. Chem. Res.* 46(26): 8846-8851.

Mirghani, M.E.S., Y.B. Che Man, S. Jinap, B.S. Baharin, and J. Bakar. 2002. FTIR spectroscopic determination of soap in refined vegetable oils. *J. Am. Oil Chem. Soc.* 79(2): 111-116.

Moser, B.R. 2009. Biodiesel production, properties, and feedstocks. *In Vitro Cell. Dev. Biol.-Plant* 45(3): 229-266.

Moser, B.R. 2008. Influence of blending canola, palm, soybean, and sunflower oil methyl esters on fuel properties of biodiesel. *Energy Fuels* 22(6): 4301- 4306.

Myers, R.H., and D.C. Montgomery. 1995. *Response Surface Methodology: Process and Product Optimization Using Designed Experiments.* New York, NY: Wiley-Interscience Publication.

Pfalzgraf, L., I. Lee, J. Foster, and G. Poppe. 2007. Effect of minor components in soy biodiesel on cloud point and filterability. *Inform supplement – biorenewable resources* no. 4, 17-21, Champaign, Ill: AOCS Press.

Sanford, S.D., J.M. White, P.S. Shah, C. Wee, M.A. Valverde, and G.R. Meier. 2009. Feedstock and biodiesel characteristics report. Ames, Iowa: Renewable Energy Group, Inc.

Selvidge, C. 2008. Biodiesel fuel blend. US Patent 11/674, 887.

Sen, A., and M. Srivastava. 1990. Outliers and influential observations. In *Regression Analysis: Theory, Methods, and Applications,* 154-179. New York, N.Y.: Springer-Verlag.

Van Gerpen J., B. Shanks, R. Pruszko, D. Clements, and G. Knothe. 2004. Biodiesel analytical methods. Golden, Colo.: National Renewable Energy Laboratory.

Van Gerpen, J.H., Hammond E.G., Johnson L.A., Marley S.J., Yu L., Lee I., Monyem A. 1996. Determining the influence of contaminants on biodiesel properties. Urbandale, Iowa: The Iowa Soybean Promotion Board.

Van Gerpen, J., and G. Knothe. 2005. Basics of the transesterification reaction. In *The Biodiesel Handbook*, 26-41. G. Knothe, J. Krahl, and J. Van Gerpen, eds. 1st Ed. Champaign, Ill: AOCS Press.

Vick, B.A., C.C. Jan, and J.F. Miller. 2004. Two-year study on the inheritance of reduced saturated fatty acid content in sunflower seed. *Helia* 27(41): 25-40.

GENERAL CONCLUSIONS

Breeding canola seed for biodiesel use requires evaluation of properties of the seeds from experimental canola lines and the resulting biodiesel. One challenge lies in biodiesel preparation when hundreds of seed lines are waiting for evaluation. The conventional preparation method is time-consuming, requires much seed and has low yields, thus was not appropriate for seed screening. Therefore, this study adopted and optimized the *in situ* alkaline TE to accelerate the biodiesel preparation. Assessing 40 samples per wk is an achievable goal, and operator time is significantly reduced through using this method. Another advantage of the *in situ* TE over the conventional TE is that this method realizes prepared biodiesel samples from very limited amounts of canola seeds, and avoids cross-contamination between lines.

In each *in situ* TE reactor, 30-40 mL biodiesel is produced. This amount is enough for most property analysis, but not the cold soak filterability which requires 300 mL biodiesel for each measurement. In order to incorporate biodiesel characterization with the *in situ* TE, effects of impurities impacting biodiesel cold soak filterability were evaluated to provide model biodiesel samples for calibration. A downscaled model of the filtration test with 25 mL biodiesel sample for each measurement was tested and calibrated.

Biodiesel is renewable and biodegradable. It shows good potential to be an alternative to petro diesel. Developing suitable canola lines for biodiesel use helps secure the feedstock supply for future plant operation. The results of this study are instrumental to developing a higher throughput screening tool for canola breeders.

FUTURE WORK

Further adjustments of the current *in situ* protocol would provide some practical benefits. Replacing potassium hydroxide with potassium methoxide as a catalyst would help reduce moisture in the reaction mixture, and likely increase biodiesel yields after optimization of reaction conditions. Water wash in the current protocol accounts for 40% of total operator time. Simplification of the water wash step would save considerable time and increase sample evaluation throughput.

Study of co-solvent use (petroleum ether or diethoxymethane) in the alkaline *in situ* TE would be worthwhile. This inclusion might reduce the methanol use and the reaction time, and meanwhile would offer higher biodiesel yields (> 95%) (See the *In situ TE* in the *Literature Review* section). The wash step should be modified accordingly when any co-solvent is used in the TE reaction.

Another promising technology to efficiently prepare canola biodiesel is the supercritical methanol process. This method achieves high biodiesel yield from lipid within several min without catalyst. Due to the increased oil solubility in supercritical methanol, the oil extraction and TE could be done simultaneously within 1 h in this process. Equipment for this process would be much more expensive due to the high pressure involved; however, it may significantly improve the throughput of biodiesel preparation.

Further validation of the downscaled model for cold soak filtration test would be desirable. It could be done by testing the model with biodiesel samples of a wider range of filterability. The downscaled model already consumes much less biodiesel than the amount required by the ASTM D 7501-09b; however, a spectroscopic technique such as FT-IR would still be worth exploring as a high throughput technique.

APPENDIX A: BIODIESEL QUALITY TESTS

Table 12. Moisture content data (ppm) of biodiesel samples prepared through iTE and CTE.

Treatment	Rep. of reaction	Value 1	Value2	Value3	Mean	Overall mean	SD
WL	R1	628	456	499	478	470	56
	R2	478	387	435	411		
	R3	522	465	521	522		
WM	R1	502	562	603	583	557	25
	R2	525	464	541	533		
	R3	563	485	547	555		
WH	R1	555	473	555	555	521	35
	R2	486	483	NA	485		
	R3	433	545	500	523		
WC	R1	262	232	NA	247	253	174
	R2	426	435	NA	431		
	R3	79	85	NA	82		
PL	R1	581	488	534	511	526	13
	R2	519	551	NA	535		
	R3	550	516	NA	533		
PM	R1	513	573	571	572	564	7
	R2	546	570	NA	558		
	R3	570	505	552	561		
PH	R1	443	603	481	462	496	37
	R2	497	482	NA	489		
	R3	525	358	546	536		
PC	R1	456	446	NA	451	180	234
	R2	42	45	NA	43		
	R3	45	49	NA	47		

A third measurement is required if the difference between first two measurements is greater than 50 ppm.

Table 13. Kinematic viscosity data (cSt) of biodiesel samples prepared through iTE and CTE.

Treatment	Rep.	Value 1	Value 2	Mean		SD
WL	R1	4.79	4.78	4.78	4.78	0.04
	R2	4.83	4.81	4.82		
	R3	4.70	4.78	4.74		
WM	R1	4.78	4.75	4.77	4.69	0.15
	R2	4.54	4.50	4.52		
	R3	4.78	4.79	4.79		
WH	R1	4.70	4.75	4.73	4.78	0.05
	R2	4.85	4.82	4.84		
	R3	4.75	4.79	4.77		
WC	R1	4.78	4.74	4.76	4.90	0.13
	R2	4.94	4.92	4.93		
	R3	5.00	5.02	5.01		
PL	R1	4.72	4.74	4.73	4.77	0.03
	R2	4.78	4.79	4.79		
	R3	4.78	4.80	4.79		
PM	R1	4.67	4.70	4.69	4.71	0.02
	R2	4.68	4.73	4.70		
	R3	4.72	4.73	4.72		
PH	R1	4.71	4.68	4.70	4.79	0.12
	R2	4.92	4.93	4.92		
	R3	4.76	4.74	4.75		
PC	R1	4.71	4.68	4.70	4.73	0.04
	R2	4.75	4.70	4.73		
	R3	4.75	4.78	4.77		

Table 14. Free fatty acid content data of biodiesel samples prepared through iTE and CTE.

Treatment	Rep.	Biodiesel g	0.1 N KOH mL	Acid value	% FFA
WL	R1	5.0113	0.48	0.54	0.27
	R2	5.0103	0.49	0.55	0.28
	R3	5.0115	0.37	0.41	0.21
WM	R1	5.0097	0.54	0.60	0.30
	R2	5.0164	0.88	0.98	0.49
	R3	5.0071	0.59	0.66	0.33
WH	R1	5.0066	0.57	0.64	0.32
	R2	5.0066	0.97	1.09	0.55
	R3	5.0135	1.00	1.12	0.56
WC	R1	5.0020	0.90	1.01	0.51
	R2	5.0456	1.07*	1.19	0.60
	R3	5.0110	0.51	0.57	0.29
	R4	5.0345	0.89	0.99	0.50
	R5	5.0134	0.54	0.60	0.30
	R6	5.0104	0.49	0.55	0.28
PL	R1	5.0012	0.38	0.43	0.21
	R2	5.0005	0.46	0.52	0.26
	R3	5.0047	0.35	0.39	0.20
PM	R1	5.0108	0.40	0.45	0.23
	R2	5.0101	0.57	0.64	0.32
	R3	5.009	0.40	0.45	0.23
PH	R1	5.0082	0.51	0.57	0.29
	R2	5.0114	0.62	0.69	0.35
	R3	5.0121	0.63	0.71	0.35
PC	R1	5.01	0.38	0.43	0.21
	R2	5.01	0.49	0.55	0.28
	R3	5.00	0.55	0.62	0.31
	R4	5.00	0.53	0.59	0.30
	R5	5.01	0.37	0.41	0.21
	R6	5.01	0.51	0.57	0.29

The value with star (*) was measured incorrectly (caused by air bubble during titration).

Table 15. Total glycerin data of biodiesel samples prepared through iTE and CTE.

Treatment	Rep.	Reading %	Dilution rate	Total Glycerin %	Mean	SD
WL	R1	0.0083	5	0.0415	0.051	0.014
	R2	0.0135	5	0.0675		
	R3	0.0089	5	0.0445		
WM	R1	0.0039	5	0.0195	0.025	0.005
	R2	0.0057	5	0.0285		
	R3	0.0051	5	0.0255		
WH	R1	0.0037	5	0.0185	0.018	0.002
	R2	0.0038	5	0.019		
	R3	0.0032	5	0.016		
WC	R1	NA	NA	0.0915	0.246	0.143
	R2	NA	NA	0.2725		
	R3	NA	NA	0.3735		
PL	R1	0.0155	5	0.0775	0.097	0.044
	R2	0.0132	5	0.066		
	R3	0.0294	5	0.147		
PM	R1	0.007	5	0.035	0.045	0.009
	R2	0.0092	5	0.046		
	R3	0.0107	5	0.0535		
PH	R1	0.0052	5	0.026	0.032	0.006
	R2	0.0074	5	0.037		
	R3	0.0063	5	0.0315		
PC	R1	NA	NA	0.117	0.158	0.065
	R2	NA	NA	0.1245		
	R3	NA	NA	0.2325		

Table 16. OSI data (h) of biodiesel samples prepared through iTE and CTE.

Treatment	Rep. 1	Rep. 2	Rep. 3	Mean	SD
WL	1.4	1.76	1.35	1.5	0.2
WM	2.5	3.15	3.65	3.1	0.6
WH	2.15	3.15	4.7	3.3	1.3
WC	4	NA	4.08	4.0	0.1
PL	1.5	1.15	1.15	1.3	0.2
PM	2.35	3	1.75	2.4	0.6
PH	2.85	2.65	3.05	2.9	0.2
PC	NA	3.08	7.53	5.3	3.2

Values indicated as NA were less than 0.5 h (unreasonable values), and were removed from analysis.

Table 17. Cloud point of biodiesel samples of two locations prepared through CTE.

Sample	Value 1	Value 2	Value 3	Mean
WC	1.1	-0.6	0	0.2
PC	-0.6	0	-0.3	-0.3

Table 18. Repeated OSI tests for ADM canola oil (sample #: 810720; OSI=10.05 h).

Rep.	1	2	3	4	5	6	7	Mean	SD
OSI (h)	9.85	9.75	9.2	10.1	9.65	10.4	10.3	9.89	0.41

Table 19. Fatty acid profiles (%) of canola biodiesel, MG gels, and SMG pellets by gas chromatography.

Fatty acid	Canola biodiesel	MG gels (MAK)	SMG pellets (SBK)
C16:0	3.8	11.0	11.9
C18:0	2.1	4.3	85.6
C18:1	67	27.4	0.5
C18:2	17.4	54.5	0.4
C18:3	8.5	0.6	ND
C20:0	ND	0.3	0.5
C22:0	ND	ND	0.3
Total SFA	5.9	15.6	98.3
Total UFA	92.9	82.5	0.9

ND: Not detected.

APPENDIX B: CENTRAL COMPOSITE DESIGN AND RESULTS

Table 20. Coded and actual levels of trace contaminants.

	Coded level		
Actual level	**-1**	**0**	**+1**
X_1: **[SMG] (% w/w)**	0.04	0.10	0.16
X_2: **[Glycerin] (% w/w)**	0.04	0.17	0.30
X_3: **[Soap] (% w/w)**	0.00	0.01	0.02

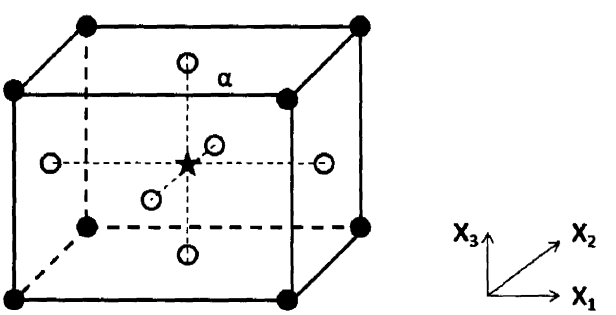

Figure 17. The illustration of the central composite design for k = 3 and α = 1. (●) factorial points; (O) axial points; (★) the central point.

Table 21. The design table for the central composite design.

Treatment	SMG	Glycerin	Soap	Replicates
1	-1	-1	-1	2
2	+1	-1	-1	2
3	-1	+1	-1	2
4	+1	+1	-1	2
5	-1	-1	+1	2
6	+1	-1	+1	2
7	-1	+1	+1	2
8	+1	+1	+1	2
9	-1	0	0	2
10	+1	0	0	2
11	0	-1	0	2
12	0	+1	0	2
13	0	0	-1	2
14	0	0	+1	2
15	0	0	0	6

Table 22. The parameter estimates (coded level of SMG, glycerin and soap) and their significance levels for the trial run of regression analysis.

Term	Coefficient	SE Coefficient	*t*	*p*
Constant	60.6	2.4	25.0	0.000*
X_1	-3.2	1.9	-1.7	0.108
X_2	7.6	1.9	4.0	0.001*
X_3	14.5	2.0	7.3	0.000*
$X_1 * X_1$	0.14	3.5	0.04	0.969
$X_2 * X_2$	3.1	3.5	0.90	0.381
$X_3 * X_3$	19.2	3.7	5.2	0.000*
$X_1 * X_2$	-7.1	2.1	-3.3	0.003*
$X_1 * X_3$	-7.4	2.1	-3.5	0.002*
$X_2 * X_3$	-4.8	2.2	-2.2	0.037*

The *p* values with star (*) indicate statistically significant. The insignificant terms were removed in the final regression model.

	A	B	C	D	E
21					
22	Variables input	Actual level (%)	Coded level		
23	$X_{1:SMG}$				
24	$X_{2:Glycerin}$				
25	$X_{3:Soap}$				
26					
27	***p***	Terms	Coeffecients	Value of terms	Contribution
28	0	***Constant***	61.5	1	61.5
29	0.108	X_1	-3.2	0	0
30	0.001	X_2	7.6	0	0
31	0	X_3	14.6	0	0
32	0.969	$X_1 * X_1$	0	0	0
33	0.381	$X_2 * X_2$	0	0	0
34	0	$X_3 * X_3$	21	0	0
35	0.003	$X_1 * X_2$	-7.1	0	0
36	0.002	$X_1 * X_3$	-7.4	0	0
37	0.037	$X_2 * X_3$	-4.8	0	0
	Prediction	***Filtration time (s)***			61.5
38					
39					

Figure 18. Prediction model for canola biodiesel filtration time (s) based on levels of trace contaminants.

APPENDIX C: FT-IR SPECTRA OF BIODIESEL AND TRACE CONTAMINANTS

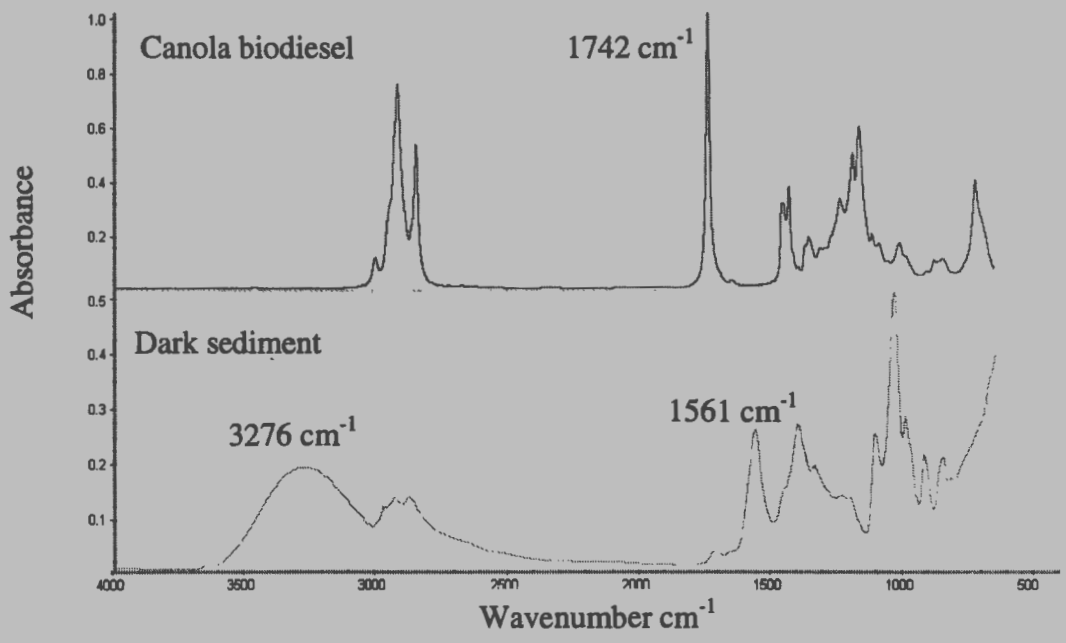

Figure 19. FT-IR spectra of neat canola biodiesel and dark sediment formed after one month storage of biodiesel.

Table 23. Characteristic peaks of biodiesel sample and some trace contaminants.

Analytes	Characteristic peaks		
	1742 cm^{-1} Ester absorption	*1561 cm^{-1}* Carboxyl ion	*3276 cm^{-1}* Hydroxide
MGs (DGs)		ND	
Soap	ND		ND
Glycerol	ND	ND	
Biodiesel		ND	ND
White bottom			ND
Dark sediment	ND		

ND: Not detected.

APPENDIX D: COLD SOAK FILTERABILITY OF CANOLA BIODIESEL

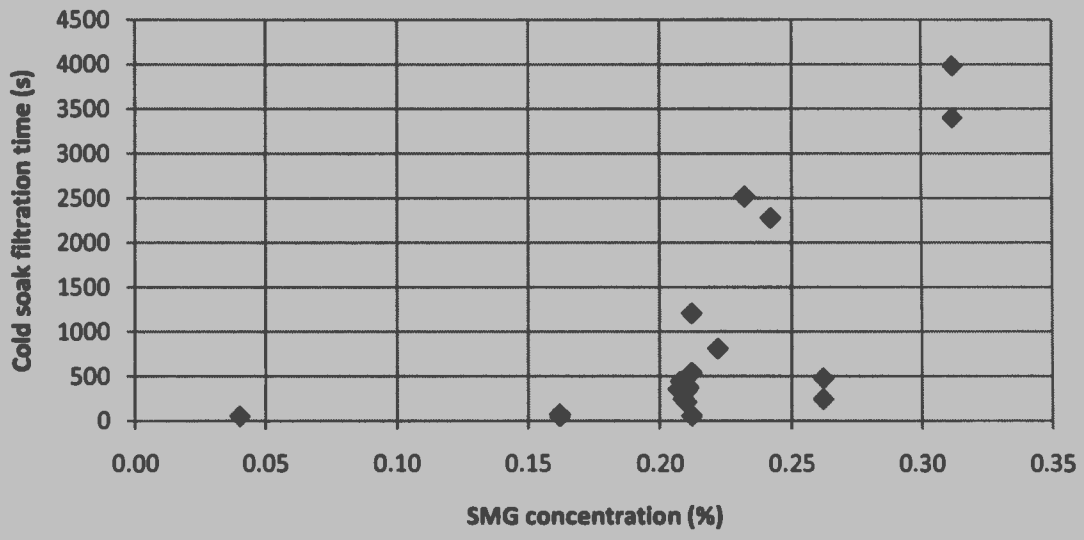

Figure 20. Relationship between cold soak filtration time of canola biodiesel and the level of saturated monoglycerides.

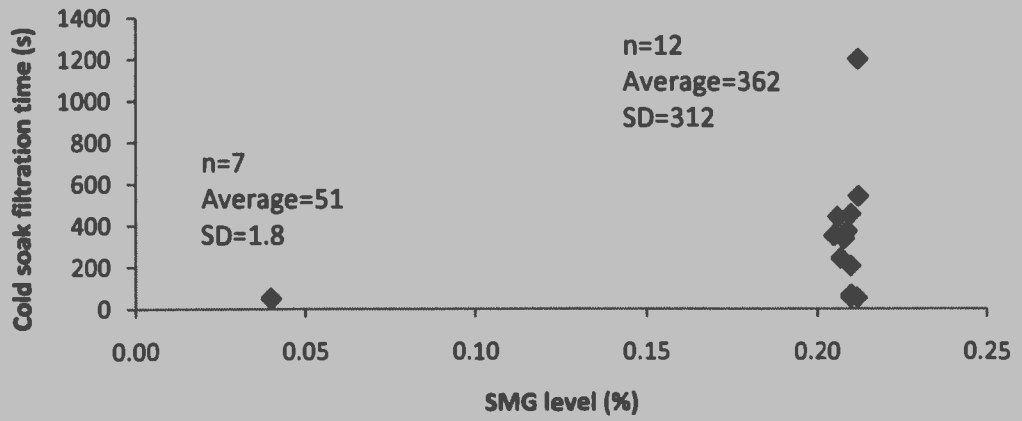

Figure 21. Cold soak filtration time of canola biodiesel tested on SMG=0.04% and 0.21%.

Table 24. Filtration times of biodiesel (25 mL and 300 mL) tested in two different filtration systems

Test Data	Sample ID	Filtration time of 25 mL biodiesel in small filtration system	Cold soak filtration time (300 mL)
8-May	1	16	55
	2	16	62
	3	17	63
	4	46	229
	5	25	104
	6	15	54
10-May	7	19	77
	8	22	89
	9	22	81
	10	63	326
2-Jun	11	22	79
	12	21	82
	13	25	90
4-Jun	14	22	84
	15	22	85

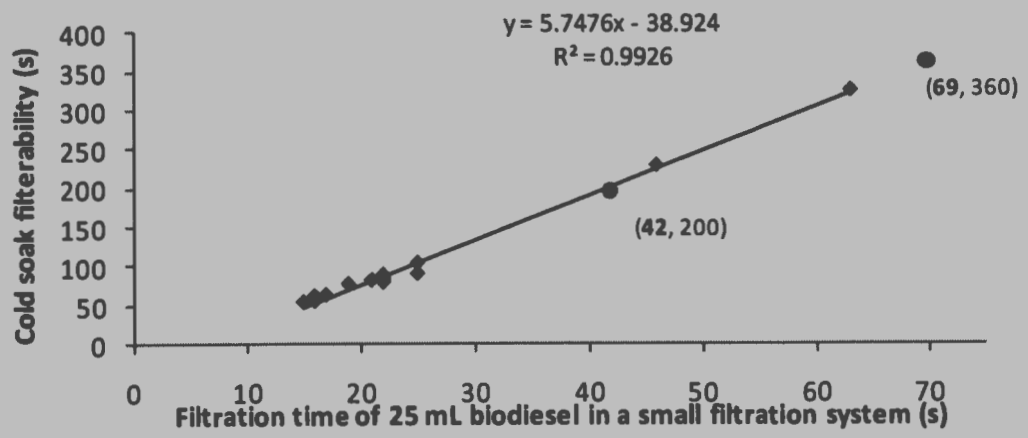

Figure 22. Downscaled model for cold soak filtration test. Cutoff values of 42 and 69 s for ASTM limit of 200 and 360 s, respectively.

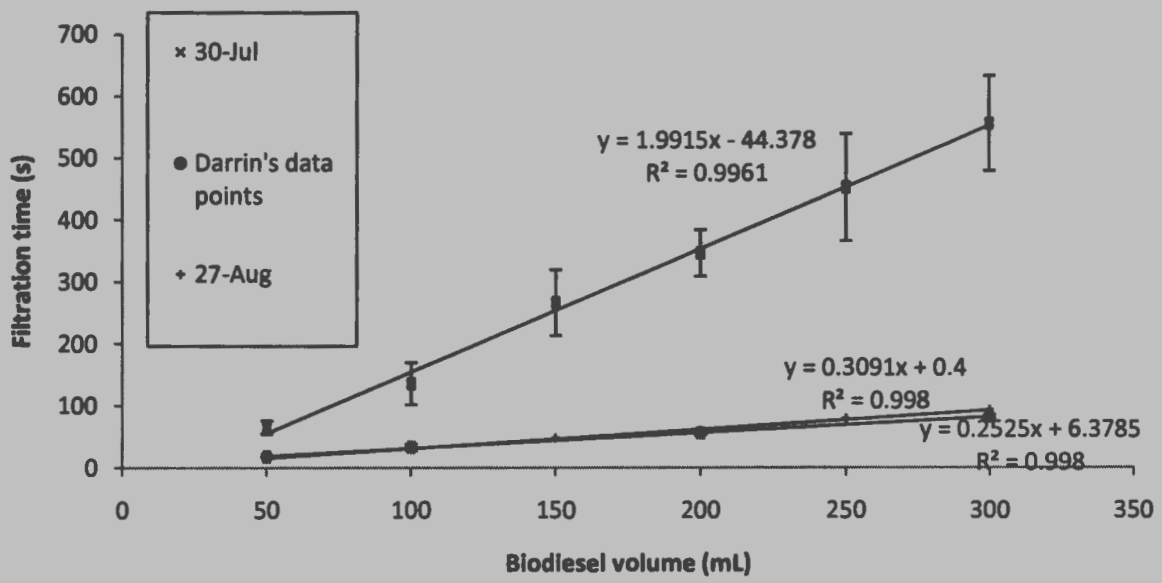

Figure 23. Relationship between filtration time and biodiesel volume based on three types of canola biodiesel.