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ORIGINAL PAPER

Implementing an In Situ Alkaline Transesterification Method for Canola Biodiesel Quality Screening

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Abstract Increasing demand for canola (*Brassica napus*) as an edible oil crop and biodiesel (B100) feedstock has encouraged genetic development for increased oil yields and expanded acreage in the US Northern Plains. Crop production environment and plant genetics influence metabolism and fatty acid composition, but the influence of this interaction on the resulting fatty acid methyl esters (FAME) is not clearly understood. The objective of this study was to develop a canola in situ transesterification (TE) method for facilitating the identification of genetic, abiotic or biotic factors impacting B100 quality, and to evaluate FAME quality properties from conventional TE (degummed oil) and in situ TE methods. In situ reactions containing 40 g canola flour conducted for 6 h at 60 °C with a 275:1:1.05 M ratio of methanol:triacylglycerol (TAG):KOH provided 80% conversion of seed lipid to FAME. Replicated reactions provided sufficient FAME volume for measuring several ASTM D6751-09 standards including cloud point, kinematic viscosity, acid value, moisture content, oxidative stability, and total glycerin, but adjustments are necessary to provide sufficient volumes for routine analysis of cold soak filtration test. The established in situ protocol would permit weekly analysis of 40 samples and the in situ TE method provides an opportunity to evaluate the impact of genetic or environmental factors on B100 quality.

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Introduction

Biodiesel is a renewable, alternative fuel suitable for use in compression ignition engines as a direct fuel replacement, and as blends with petroleum [1–4]. ASTM D 6751-09 defines biodiesel (B100) as mono-alkyl esters of long chain fatty acids, derived from vegetable oils or animal fats [2]. For industrial processing of vegetable oil to biodiesel, chemical transesterification (TE) is routinely performed with methanol and an alkaline catalyst that yields fatty acid methyl esters (FAME) and glycerol [3]. Conversion of vegetable oils to biodiesel may be accomplished through conventional TE methods where biodiesel conversion results from a two-step process involving oil extraction and refining, followed by TE and post-reaction FAME purification. An alternative processing method is the direct TE of seed lipids (in situ TE) without prior oil refining, which eliminates the cost and time of oil extraction [5–7]. Harrington and D'Arcy Evans [5] characterized acid catalyzed FAME production in sunflower with in situ or conventional methods, and both methods yielded high conversion of seed lipids to FAME. More recently, Haas and Scott examined alkaline in situ TE of soybean flakes and a reduction in seed moisture content provided >97% FAME conversion of seed lipid [8]. Similarly, high conversion of lipid to FAME has been reported in cottonseed [9], and rice bran [10], among others, but in situ TE reactions utilizing canola or rapeseed have not been previously reported.

The parent oil's fatty acid composition has a major influence on biodiesel quality [11, 12]. Oils containing high levels of unsaturated fat may have improved low

temperature performance, but experience decreased oxidative stability when compared to oils possessing high saturated fat contents. When compared to biodiesel produced from palm or soybean, biodiesel produced from canola may have improved low temperature performance. The improved performance may be attributed to canola's low saturated fat (<7%) and high unsaturated fatty acid composition (63 and 23%, mono and polyunsaturated fatty acid contents, respectively). In the United States, canola production is primarily confined to the Northern Great Plains, which may be attributed to canola's adaptation to cool temperature. To support the increasing demand for food use and additional biofuel markets, efforts are ongoing to expand canola acreage through improved genetics and agronomic practices. A method is needed to rapidly identify potential new canola varieties not well suited as a biodiesel feedstock. If canola production acreage is expanded, canola may be exposed to increased variability in temperature and precipitation. High temperature stress (>30 °C) during canola flowering is closely associated with increased seed protein concentrations, decreased oil content, and increased saturated (16:0, 18:0) fatty acid concentrations [13, 14]. Although plant genetics and growing environment impact fatty acid composition, reports documenting the impact of genetic variation within a crop species on ASTM D6751 (B100) quality specifications are limited. In growth chamber studies, esters derived from two sunflower cultivars across a range of temperatures revealed a large variation in ester content yield and cloud points ranged from 3 to −5.5 °C [5].

Characterizing fatty acid profiles among varying canola genotypes across contrasting growing environments may provide an indicator of cloud point or temperature of wax crystallization, but this characterization may not relate to overall operability of biodiesel at low temperature as other constituents may interact in storage to decrease operability. Assessment of cold filter plugging point or cold soak filterability tests were developed to more effectively predict operability at low temperatures. Extracting, refining, and transesterifying oil from individual seed samples via conventional methods is too time-consuming for high throughput evaluation of breeding materials. To provide sufficient amounts of biodiesel for examining storability and cold temperature operability, a rapid method of supplying quantities of biodiesel sufficient for laboratory tests is necessary. Establishing a rapid method of FAME production may also aid in the identification of agronomic, abiotic or biotic interactions impacting oil feedstock and FAME quality. Therefore, primary objectives of this study were to identify in situ alkaline TE process conditions that would provide adequate FAME yields for screening canola lines, and to evaluate FAME quality properties from both conventional TE and in situ methods. The potential throughput for each TE process for canola screening was examined.

Materials and Methods

Materials

Canola seed samples were obtained from Langdon, ND, 2008. Seed was cleaned according to USDA-GIPSA methods [15], and oil content prior to TE was determined from intact seeds by NIR spectroscopy (DA 7200, Perten Instruments, Springfield, IL). Flaked canola was kindly provided by ADM, Velva, ND. All reagents were of analytic reagent grade and were purchased from EMD Chemicals (Gibbstown, NJ).

Conventional Transesterification

Seed samples (2 kg) at 7% moisture were screw pressed (Komet Oil Expeller S 87G, IBGMonforts, Germany). The expeller was preheated to 70 °C, and was equipped with an R8 screw operated at minimum speed (20 rpm), with a 6 mm-opening die. Pressed oil was clarified and degummed with citric acid as described by Vargas-Lopez et al. [16]. Degummed oil samples were converted to biodiesel using a molar ratio of 6:1:0.2 of methanol:triacylglycerol (TAG):KOH at 60 °C for 1 h with mechanical mixing. After the reaction was completed, the mixture was transferred to a separatory funnel and the lower glycerol phase was discarded. Methanol was recovered from the ester phase under vacuum (65 °C, 80 kPa, 1 h). The crude FAME were neutralized with acetic acid, and water washed three times with distilled water and dried under vacuum (95 °C, 80 kPa, 1 h). The refined FAME samples were transferred to amber glass bottles and stored at room temperature until quality analysis. Quality analyses were conducted within 1 month of processing.

In Situ Transesterification

Seed Preparation

Canola seed was ground at room temperature (defined as cold ground canola) with a coffee grinder and typical particle size of the canola flour 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 for 3 h or until 1.0% dry basis moisture content was obtained.

General Reaction Conditions and Washing

Canola flour with an oil equivalent of 19.2 g was added to 500 mL Erlenmeyer flasks. Methanol volume, KOH concentration, and reaction time varied depending upon experiment (refer to "Experimental Design" for details). After addition of methanolic KOH, flasks were sealed with rubber stoppers and reactions were conducted at 60 °C in a shaker water bath at 200 rpm. After the reaction was complete, the samples were vacuum filtered (Whatman #4, 80 kPa vac). The post-reaction flour was washed in triplicate with 60 mL methanol, and the methanol washes were combined with the liquid filtrate. The pooled liquid filtrate was transferred to pre-weighed 13 × 33 cm aluminum baking pans and the methanol was removed by vacuum oven drying (60 °C, 80 kPa) for 12 h. Following methanol removal, crude FAME weights were recorded upon transfer to preweighed vials. The crude FAME pH was neutralized with a few drops of HCl or acetic acid, and samples were washed sequentially with 1 vol. distilled water, 0.3 vol. of 0.5 M NaCl, 0.1 vol. of 0.5 M NaCl, and 1 vol. distilled water with centrifugation at 3500×*g* for 15 min between washes. FAME samples were dried with the addition of 10% w/v anhydrous $MgSO_4$. The refined FAME samples were transferred to amber glass bottles and stored at room temperature for up to 1 month until quality analysis.

Seed, Oil and FAME Quality Analysis

Oil (containing mono-, di-, TAG, or unrecovered FAME) was extracted from canola flour (pre and post TE) with *n*-hexane using accelerated solvent extraction (ASE 200, Dionex Corp, Sunnyvale, CA). For oil extraction, 4 g flour (oven dried 70 °C) was milled in a coffee grinder with 2.5 g diatomaceous earth (DE), and samples were loaded into 11-mL stainless steel cells. Any remaining extraction cell void volume was filled with DE prior to extraction. Extractions were performed at 100 °C, 6.7 MPa with a 5 min equilibration time and three 10 min static cycles having a 100% flush volume and 60-s purge time. The solvent containing extracted oil was collected in preweighed vials, and solvent was evaporated to dryness with a stream of dry air (−70 °C dew point). Extracted samples were air dried, and reground for a second extraction and the total oil recovery from the two extractions was recorded. Fatty acid profiles of canola oil and FAME were determined by gas chromatography according to methods described by Espinoza-Pérez et al. [17].

Kinematic viscosity (40 °C), acid value, cloud point, cold soak filtration, moisture content, oxidative stability index (OSI) from the refined FAME were analyzed according to tests included in the ASTM D6751-09 for B100 [2]. Total glycerol was quantified by the SafTest for total glycerin according to the manufacturer's recommendations (MP Biomedical, Solon, OH).

Experimental Design and Statistical Analysis

Experiment: 1 and 2

The impact of methanol volume, KOH catalyst concentration, and reaction time on FAME yield and quality was evaluated. The experimental design was a randomized complete block arranged as a 4 × 5 factorial replicated three times. Reagent levels included four KOH concentrations (0.06, 0.08, 0.09, and 0.10 N) and five levels of methanol (180, 210, 240, 270, 300 mL), and the reactions were carried out for 16 h at 60 °C. Utilizing the reagent conditions from this experiment that yielded the greatest crude FAME, a second experiment was conducted that evaluated the impact of reaction time on FAME yield and quality. Eight reaction times at 60 °C were evaluated (1, 2, 4, 6, 8, 10, 12, and 16 h), and the experiment was replicated four times.

Experiment: 3

The impact of TE method on FAME quality was characterized. FAME quality was evaluated from canola samples obtained from Langdon ND, 2008, processed by both in situ and conventional alkaline TE methods. Each TE method was replicated three times.

Statistical Analysis

Data were analyzed using the general linear models (GLM) procedure [18]. An *F*-protected LSD ($P \leq 0.05$) was calculated for comparisons of main effect means. Significant differences ($P \leq 0.05$) between means of two way and higher order interactions were determined as twice the standard error of the mean [19].

Results and Discussion

Plant genetics and production environment influence metabolism and fatty acid composition, but the influence of genetic, agronomic and/or environmental factors on fatty acid metabolism and the resulting FAME (B100) is not clearly understood. Providing a method for rapidly producing and quantifying biodiesel from individual breeding lines may help rapidly assess genetic variation in biodiesel quality and improve our understanding of environmental or agronomic factors responsible for alterations in B100 quality. The primary objective of this research was to identify in situ TE process conditions that would provide

adequate bench-scale FAME yields for rapidly screening canola breeding lines.

Based on previous work reported on soybean in situ TE reactions, where decreased soy flake moisture content allowed for reduced methanol volumes and enhanced FAME recovery [8], experiments with canola were conducted at low seed moisture content (<1%). Preliminary research was conducted to identify reaction variables for additional optimization experiments (data not shown). Reaction variables consisted of alkali choice (KOH and NaOH) ranging from 0.04 to 0.15 N, reaction temperature (25, 50, and 60 °C), and reaction duration (1.5, 4 and 16 h). Fame yields at room temperature were 30% greater using KOH as a catalyst when compared to NaOH across similar molar concentrations and methanol volumes. Similarly, residual oil content from post reaction canola flour was 3.5-fold lower from reactions implementing KOH when compared to NaOH. The impact of alkali catalyst choice on FAME yields was reduced at reactions conducted at 60 °C, where NaOH catalyzed reactions yielded approximately 93% of the recovered FAME provided by KOH at similar molar concentrations. The explanation for the decreased FAME yields from sodium hydroxide catalyzed reactions is not clear. Singh et al. [20] evaluated conventional biodiesel production from canola oil using four alkaline catalysts (sodium hydroxide, sodium methoxide, potassium hydroxide, potassium methoxide) at different catalyst concentrations and methanol to oil molar ratios. Averaged across catalyst concentration, potassium catalysts gave higher biodiesel yields (82.8%) than sodium catalysts (75.3%). Methoxide catalyst provided greater yields (90.5%) than hydroxide catalysts (79.1%) [20]. The impact of methoxide catalyst on FAME yields is further discussed below. In addition to a reduction in FAME yields observed with NaOH catalysis, the soap content was approximately 25% higher than reactions conducted with KOH. Subsequent reactions incorporated KOH. Final KOH concentrations ranging from 0.07 to 0.1 N KOH provided the greatest crude FAME yields and lowest residual oil content at 60 °C.

Reactions conducted at 50 °C provided 50 and 60% theoretical maximum FAME yields at 1.5 and 4 h, respectively, and crude yields attained 78% of the theoretical maximum after 16 h. To obtain our objective of rapidly evaluating multiple samples, FAME recovery was evaluated across several process conditions utilizing multiposition hot plate magnetic stirrers, impeller mixers, or orbital shaking incubators. A multiple hot plate magnetic stirrer combination did not provide adequate mixing and temperature control, and incorporating impeller mixers would be costly to accommodate high sample throughput. An inexpensive shaking incubator would accommodate eight individual reactions (500-mL flasks), and temperature control was easily maintained. Additional preliminary tests conducted in a shaker bath evaluated sample mixing and volume requirements. A 500-mL Erlenmeyer flask was shown to accommodate 40 g of processed canola flour and a 180-mL minimum volume of methanolic KOH was required to ensure sample suspension at 200 rpm.

A series of experiments were carried out to further optimize in situ reaction conditions by assessing the impact of methanol volume and KOH catalyst concentration on crude FAME yields, post-reaction residual oil and crude glycerol content (Fig. 1). Reactions were conducted in 500-mL Erlenmeyer flasks containing cold ground canola flour with an oil equivalent weight of 19.2 g, and all reactions were conducted for 16 h at 60 °C. Crude FAME yields were significantly impacted by KOH concentration

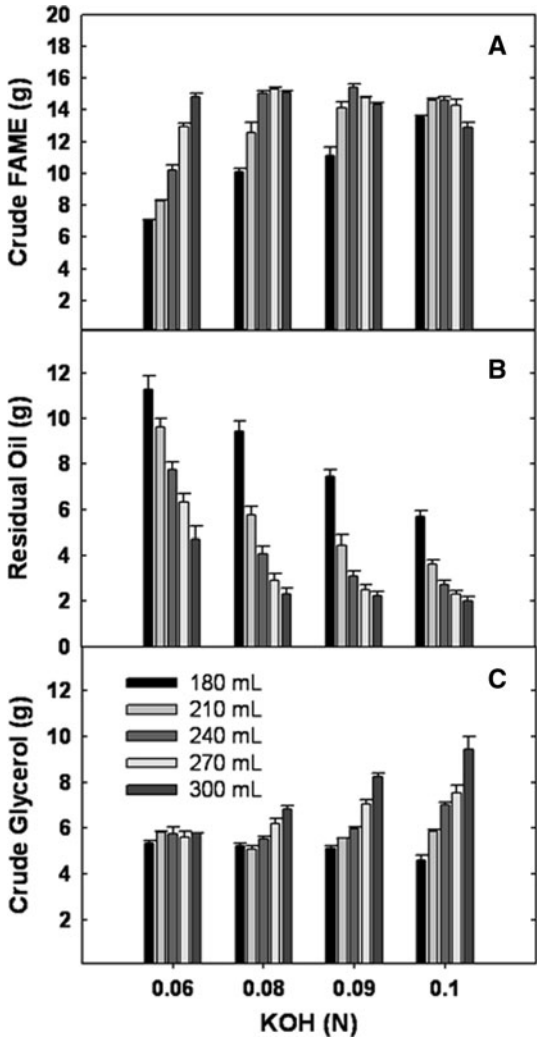

Fig. 1 Impact of methanol volume and KOH concentration on recovered crude FAME (**a**), post-reaction residual oil (**b**), and glycerol (**c**) from in situ transesterified canola. Reactions contained 40 g canola with an oil equivalent of 19.2 g and error bars represent SEM ($n = 4$)

and methanol volume and the interaction of the two variables (Fig. 1a). Highest FAME yields were obtained with 0.09 N KOH and 240 mL methanol (15.4 g), and with 0.08 N KOH and 270 mL of methanol (15.3 g). Yields obtained with 300 or 240 mL of 0.08 N closely followed with yields of 15.1 and 15.0 g, respectively (Fig. 1a). The impact of methanol volume on FAME yield varied across catalyst concentration. At reactions conducted with 0.06 N KOH, an incremental increase in methanol volume was accompanied by a significant increase in FAME yield and FAME yield nearly doubled when reaction volume increased from 180 to 300 mL. In reactions conducted at 0.08 and 0.09 N KOH, an increase in methanol volume from 180 to 240 mL increased FAME yields 49 and 38%, respectively. Increasing methanol volume above 240 mL did not increase FAME yields for 0.08 and 0.09 N KOH reactions, and in the case of 0.09 N KOH, slightly decreased yields. However, yields at 300 mL reactions remained 50 and 30% higher than from the 180-mL reactions. In contrast, increasing methanol volume did not significantly improve yields from reactions conducted with 0.1 N KOH, as FAME yields decreased slightly at 300 mL (13.5 g) when compared to 180-ml reaction yield (12.5 g). Refined FAME representing all 20 methanol-KOH treatment combinations met the ASTM D 6751 specification for acid number, total glycerin, moisture content, kinematic viscosity, and oxidative stability index (data for individual treatments not shown).

Post reaction residual oil contents and the weight of crude glycerin were determined to further evaluate in situ reaction efficacy. Residual oil content containing unreacted TAG and unwashed FAME was significantly impacted by methanol volume and catalyst concentration (Fig. 1b). Increasing methanol volume may have aided the dispersion of reactants and products, resulting in decreased residual oil content across all alkali concentrations. FAME recovery reflected residual oil contents at 0.06 N alkali concentrations, but FAME levels from 0.08, 0.09 and 0.1 N KOH treatments reached a plateau at which increases in methanol volume did not enhance FAME recovery, although residual oil content was reduced. One hypothesis is that at higher alkali concentrations excessive soap formation may have contributed to increased crude glycerol losses (Fig. 1c). Averaged across methanol treatments, a 28% increase in crude glycerin was observed as alkali concentration increased from 0.06 to 0.1 N. With reactions conducted at 0.06 N KOH, crude glycerin recovery was not impacted by methanol volume, but crude glycerin increased with increasing methanol volume from reactions containing 0.08, 0.09, and 0.1 N KOH concentrations.

For the two treatments providing the largest FAME recovery, no significant difference in residual oil content was detected, but crude glycerin from the 240-mL reaction at 0.09 N KOH (6 g) was significantly lower than that from the 270-mL reaction containing 0.08 N KOH (6.4 g). Based on the significant difference in crude glycerin in addition to the decreased time of methanol recovery for the 240 mL volume, subsequent reactions implemented the following reaction conditions: 240 mL methanol and 0.09 N KOH, or a methanol:TAG:KOH molar ratio of 275:1:1.05. The methanol ratio was slightly larger than from alkaline in situ reactions conducted with soy flakes (227:1) [8].

A time course evaluation of total FAME and residual oil recovery was conducted at 60 °C (Table 1). Based on seed TAG content, the theoretical maximum yield of crude FAME is 19.2 g. The highest FAME recoveries were obtained after 10 h, but were approximately 80% of the theoretical maximum. Increasing reaction duration past 10 h did not significantly increase crude FAME recovery, but did significantly decrease residual oil content (Table 1). Long reaction times likely increased conversion to soap. The low FAME yields in our study may be attributed to factors impacting oil miscibility with methanol. The TE reaction is considered a heterogeneous two-phase system where a non-polar TAG phase is immiscible with a polar methanol phase. Factors improving the miscibility of the reactants such as increased mixing speed, use of cosolvents, or ultrasonication enhanced FAME conversion [9, 21, 22]. Cottonseed in situ TE reactions conducted with mechanical mixing (600 rpm) or use of ultrasonication provided >95% ester conversion [9]. In our experiments, a 200 rpm shaking speed permitted suspension of the canola flour in methanol, and a higher shaker speed was not available. Increasing shaking speed may have enhanced

Table 1 Impact of reaction time on crude FAME and residual oil recovery

Reaction time[A] (h)	Crude FAME (g)	Residual oil[B] (g)
1	9.8 a	8.2 a
2	11.5 b	7.6 a
4	13.7 c	4.7 b
6	14.8 d	3.4 c
8	15.1 d	2.8 d
10	15.5 e	2.2 e
12	15.7 e	1.8 f
16	15.8 e	1.5 g

Data represent the mean of four reactions and means within each column followed by the same letter are not significantly different at $P \leq 0.05$

[A] Reactions were conducted with a methanol:TAG:KOH molar ratio of 275:1:1.05 at 60 °C

[B] Residual oil consists of unreacted TAG and unrecovered FAME from post reaction canola flour

FAME conversion by increasing mass transfer of oil to the methanol interphase.

Another explanation for decreased FAME yields is large seed particle size may have hindered access of methanolic KOH to seed lipids. Investigating canola oil extraction with hexane, So and MacDonald [23] concluded that flake thickness had a great impact on extraction rate. An evaluation of soybean acid alcoholysis, the amount of soybean oil extracted by alcohol increased 78 to 98% as seed particle size decreased from 1 to <0.5 mm [24]. In our in situ experiments, approximately 87% of the cold ground seed particles passed through a 0.85-mm sieve opening but only 30% passed through a 0.30-mm opening. In contrast to these particle sizes, the typical thickness of flaked canola may range from 0.20 to 0.40 mm. To further investigate the impact of seed preparation on FAME conversion, the yield of FAME produced from conditioned flaked canola and cold ground canola flour was evaluated at 1, 2, 4, 6, and 16 h reaction times (Fig. 2). Oil contents for flaked and cold ground canola were 47.7 and 48% as determined by solvent extraction. FAME yields from flaked canola were 27 and 20% greater than those from cold ground canola at 1-h and 2-h reactions, but were only 6% greater at 4 h, and no differences in FAME yield were noted at 6 and 16 h between the seed preparation treatments (Fig. 2). Although flaked canola had significantly greater FAME yields at reaction times <4 h, seed preparation treatment did not impact total FAME recovery, suggesting other factors are limiting FAME conversion. Poor solubility of oil in methanol may have contributed to low FAME conversion as oil solubility is affected by alcohol selection. In situ reactions with ethyl, propyl, or butyl-alcohol increased soybean oil solubility nearly fourfold and significantly

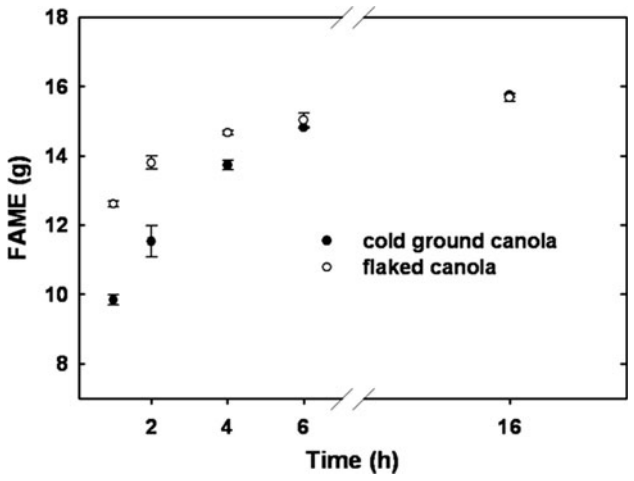

Fig. 2 Impact of seed preparation treatment (cold ground or flaked) on in situ FAME yields over varying reaction time. Reactions contained 40 g canola with an oil equivalent of 19.2 g and *error bars* represent SEM ($n = 4$)

enhanced ester conversion compared with methanol under acid-catalysis [24]. In contrast, under alkaline catalyzed in situ TE, soybean ester contents were similar with methanol, ethanol, and isopropanol as visualized by TLC [7]. Canola in situ reactions were evaluated using methanol and lowest residual oil content was 1.5 g or approximately 8% of the initial oil content at 16 h. Incorporating a less polar alcohol in combination with increased mixing speed would likely increase FAME conversion.

Although acquiring a methoxide catalyst was not cost effective for our laboratory screening protocol, the prospect of achieving much higher than 80% FAME yields warranted a further evaluation of methoxide catalyst. Reactions containing equivalent moles of KOH or sodium methoxide ($NaOCH_3$) catalyst were compared at the optimized methanol:TAG:catalyst molar ratio (275:1:1.05). Moisture content of the catalysts was determined with Karl Fischer coulometric titration and the methanolic KOH moisture content was fourfold greater than the commercial $NaOCH_3$ solution. However, no significant difference in FAME yield were noted between KOH and $NaOCH_3$ treatments, and the crude FAME yields were still only approximately 80% of the theoretical maximum. Reactions were conducted at a catalyst concentration optimized for KOH, and a further optimization of $NaOCH_3$ concentrations may have improved FAME conversion and recovery.

The final objective of the study was to evaluate the impact of the TE method on FAME quality. In an effort to establish a screening protocol for identifying canola with superior biodiesel quality (i.e., cold flow properties, oxidative stability), the ability to discriminate changes in quality associated with feedstock (genetics), apart from processing is imperative. For this experiment, bulked canola samples were obtained and alkaline TE processing was conducted from screw pressed degummed oil, and directly from in situ TE. Several quality parameters were evaluated from FAME produced via in situ or conventional alkaline TE methods (Tables 2, 3), and all parameters met the ASTM D6751-09 specification for B100. Table 2 presents the principal fatty acid composition of screw pressed canola oil, FAME derived from pressed oil, and FAME

Table 2 Principal fatty acid composition of canola oil and FAME produced from conventional or from in situ alkaline transesterification methods

Fatty acid	Canola oil	Conventional	In situ
Palmitic (16:0)	3.9	3.8	4.6
Stearic (18:0)	1.1	2.1	1.7
Oleic (18:1)	64.4	67.0	65.1
Linoleic (18:2)	20.4	17.4	20.0
Linolenic (18:3)	9.6	8.5	8.6

Table 3 Comparison of refined canola FAME derived from in situ or conventional alkaline transesterification processing

Property	Assay method[a]	Biodiesel process		Limits	Units
		In situ	Conventional		
Acid number	ASTM D664	0.09	0.203	0.5 max	mg KOH/g
Kinematic viscosity	ASTM D 445	4.7	4.8	1.9-6.0	mm^2/s
Water and sediment	ASTM D 2709	0.037	0.022	0.050 max	vol%
Oxidative stability	EN 14112	7.96	4.48	3 minimum	h
Total glycerin	SafTest™	0.005	0.202	0.240 max	wt%
Cloud point	ASTM D2500	−0.5[b]	−1.0	Report	°C
Cold soak filtration	ASTM D6751	67[c]	73	200	s

[a] The test method was conducted according to ASTM D6751 for biodiesel (B100) except for total glycerin which was assayed according to SafTest™ manufacturer's recommendation (MP Biomedical, Solon, OH). Data represent the mean of three replicate reactions

[b] For in situ cloud point analysis, FAME from the three replicate reactions was pooled to obtain the required 40 mL test volume; test was not replicated

[c] For in situ cold soak filtration test, FAME from additional reactions were pooled to obtain the required 300 mL test volume; test was not replicated

derived via in situ TE method, and the fatty acid composition did not differ markedly among the samples. TE process did not influence kinematic viscosity, water and sediment, and cloud point value, or cold soak filtration times, but FAME processed via in situ methods had increased oxidative stability, and decreased acid number and total glycerin when compared to FAME derived from conventional processing. Variation in OSI values could be attributed to natural antioxidant concentrations. By quantifying tocopherol levels, Haas and Scott [8] speculated soy biodiesel oxidative stability from in situ TE would be similar to or higher than from biodiesel derived from refined oil. FAME from in situ TE soy flakes had 76% higher tocopherol levels than conventional soy FAME, but OSI values were not reported. Another explanation for the contrasting OSI values is that contrasting methods of moisture removal decreased the stability of conventional processed FAME. In the conventional TE process, moisture was removed with heat under vacuum, whereas moisture removal of in situ derived FAME was from addition of anhydrous $MgSO_4$. To test this hypothesis, additional conventional TE reactions were conducted incorporating anhydrous $MgSO_4$ into the FAME refining method. Implementing the chemical drying agent increased conventional OSI from 4.48 to 8.68 h, a level similar to the OSI value obtained from in situ TE reactions (7.96 h). The impact of drying process on OSI is in agreement with other reports that oxidative induction time (OIT) is inversely related to the heating degree [25]. Corn biodiesel dried with anhydrous $NaSO_4$ had increased OIT when compared with biodiesel samples that had moisture removed with heat or with heat under vacuum [25].

Although in situ TE provided FAME with quality similar to conventional processed FAME, the small volume of FAME produced by our method represents a major challenge for implementation of a germplasm screening tool. The standardized reaction conditions (40 g seed, 19.2 g oil) conducted in 500 mL flasks yielded approximately 17.5 mL FAME (assuming 2% loss in refining). Although acid number, total glycerin, oxidative stability, and kinematic viscosity could be determined from this sample, the volumes required for the cold soak filtration (300 mL) and cloud point (40 mL), plus additional volume for replicate samples to ensure appropriate statistical analyses far exceeds the sample. In addition, if the cold soak test was performed in triplicate, the amount of seed required for conventional TE processing (screw pressing, degumming, TE) would be in excess of 2 kg, a quantity unlikely available from advanced breeding lines. Regardless of TE process, modification of the cold soak test is necessary to accommodate the limited FAME.

Our objective was to identify a protocol that would rapidly provide bench-scale quantities of FAME suitable for analysis of quality pertaining to cold weather performance and storability. A flowchart documenting a proposed screening protocol is highlighted in Fig. 3. Prior to initiation, seed oil content is determined with NIR spectroscopy, and samples are ground and oven dried (70 °C) to <1% moisture. To obtain sufficient quantities of FAME for cloud point analysis, three replicate flasks will be initiated for each canola sample and pooled for analysis. Current shaker capacity is thirty-two 500-mL flasks, and three replicate reactions per sample would yield 10 samples day^{-1}. Assuming 4 day/week are devoted to reaction prep and the fifth to final washing, clean up, and analysis, a total of 40 unique samples would be analyzed weekly. In contrast to in situ TE output, under current capabilities the conventional TE method permits weekly analysis of 12

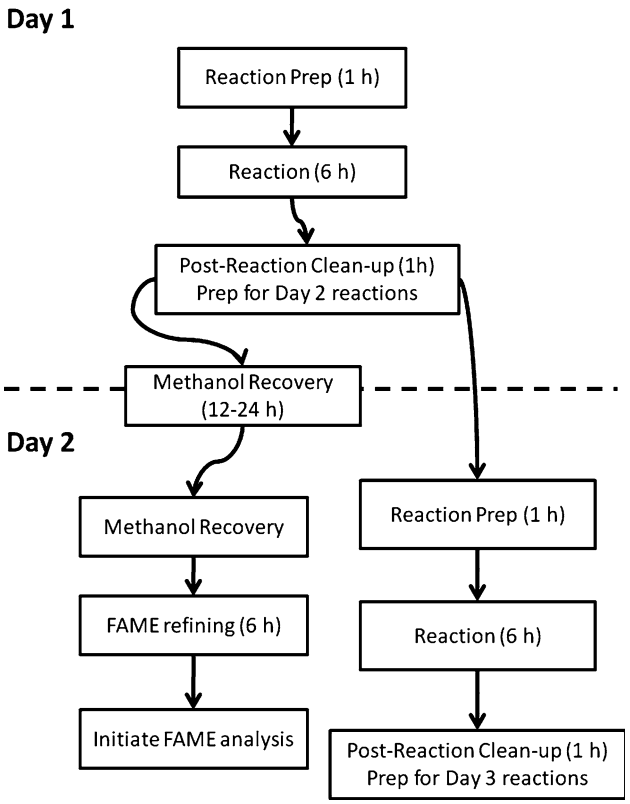

Fig. 3 Proposed model of canola FAME screening with in situ TE processing. Current lab capacity permits weekly analysis of 40 unique samples

samples. In addition, preparation time for seed conditioning, screw pressing, and oil refining require additional inputs which favors implementation of in situ processing. Therefore, in situ TE appears to be a promising method to evaluate the impact of genetic or environment effects on B100 quality.

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