EVALUATING THE FEASIBILITY OF BIODIESEL PRODUCTION FROM *CAMELINA SATIVA*

by

Jie Yang

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
July 2016

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Camelina sativa has recently attracted great interest as an energy crop for biodiesel production in North America. To assess the feasibility of biodiesel production from camelina grown in Nova Scotia, fuel properties of camelina biodiesel, alkali-catalyzed transesterification process, crude camelina biodiesel purification and improvement on the oxidative stability of camelina biodiesel were investigated in the present study. A high biodiesel yield (about 96 wt. %) was achieved at optimal reaction conditions. The crude camelina biodiesel can be purified using fiber-based biosorbents, such as wood sawdust and shavings. Most of the fuel properties were in agreement with the standards specified in the ASTM D6751 and EN 14214. The inherently poor oxidative stability of camelina biodiesel can be improved to a satisfactory level by adding 1500 ppm of antioxidant, TBHQ. The research presented herein has demonstrated a high feasibility of biodiesel production from *Camelina sativa* oil.

LIST OF ABBREVIATIONS USED

Adj R2	adjusted R-square
ANOVA	analysis of variance
AOAC	association of official analytical chemists
AOCS	American oil and chemistry society
ASTM	American Society for Testing and Materials
B100	pure biodiesel
BaCl2	barium chloride
BD	BD-Zorb
BHA	butylated hydroxyanisole
BHT	2,6-Di-tert-butyl-4-methylphenol
Ca	calcium
CaSO4	calcium sulfate
CCD	central composite design
CN	cetane number
CP	cloud point
CSFT	cold soak filtration test
DAG	diglyceride
EN	European Union
FAME	fatty acid methyl ester
$FeSO4 \cdot 7H_2O$	ferrous sulfate
FFA	free fatty acid
FID	flame ionization detector
FStG	free steryl glucosides
F-value	Fisher F-test value
GC	gas chromatograph
GHGs	greenhouse gases
ha	hectare
IP	induction period
K	potassium
KOH	potassium hydroxide

lb	pound
MAG	monoglyceride
Mg	magnesium
mM	mmol/L
Na	sodium
NaOH	sodium hydroxide
OSI	oxidative stability index
P	phosphorus
ppm	parts per million
PrG	propyl gallate
PV	peroxide value
rpm	revolutions per minute
RSM	response surface methodology
S	sulfur
SD	sawdust
TAG	triglyceride
TBHQ	tert-butylhydroquinone
WS	wood shaving

ACKNOWLEDGEMENTS

I would like to thank and express my greatest appreciation to my supervisor, Dr. Quan (Sophia) He, for her professional, scientific and continuous guidance and support throughout my MSc study. I really appreciate her encouragement, trust and inspiration that greatly motivated me to work hard, act efficiently and accomplish more. I believe I would embed those nice personality and positive attitude to my life and make further progress in the future.

I am also very grateful to my supervisory committee members Dr. Kenneth Corscadden and Dr. Claude Caldwell for their technical advice and support for my MSc project. Their contributions to this research are greatly appreciated.

I would like to thank Dr. Haibo Niu, Jili Li, Mandi Wilson, Chris Nelson, Scott Read, Yougui Chen, David Gibson and Bo Fan and other faculty members and staff as well, for their help and contributions to this research. At last but not least, I would like to show my appreciation to my families for their continuous encouragements and support that allowed me to pursue my goals at scientific research area and in return to contribute myself to investigate renewable energy for our society.

CHAPTER 1: INTRODUCTION

1.1 Background

Over recent decades, the growth of the world's population and industrialization have led to a dramatically increasing consumption of petrol fuels, which has resulted in a significant decline of petroleum reserve. Meanwhile, extensive usage of fossil fuels has also caused air pollution and growing concerns over global warming due to the emissions of greenhouse gasses. From a socioeconomic point of view, the political environment in the greatest oil exporting regions is also unstable. These combined factors are driving researchers and industrial practitioners to develop renewable and sustainable fuel alternatives (Banković-Ilić et al., 2012). Biofuels have recently attracted great interest as one of the promising substitutes for petrol fuels. It has been estimated that biofuels will make up 80% of the overall liquid fuels growth in the United States during 2010-2035 (International Energy Statistics - EIA).

Biodiesel is one such biofuel that is comparable to conventional petrodiesel and is compatible with various applications, such as trucks and automobiles, farm vehicles, stationary power and heat generation. It is renewable, environmentally friendly, biodegradable, and can be directly used in compression diesel engines without significant modifications (Graboski and McCormick, 1998; Leung et al., 2010). Biodiesel is typically defined as a mixture of fatty acid alkyl esters obtained by employing the alcoholysis of triglycerides (TAGs) from vegetable oils, animal fats and even waste cooking oils in the presence of a catalyst, and this process is named transesterification. Fig. 1.1 shows the reaction pathways of transesterification that actually is a sequence of three consecutive and reversible reactions (Veljković et al., 2012); Di- and Monotriglycerides (DAG and MAG, respectively) are formed as intermediates during transesterification process.

Currently, more than 95% of biodiesel worldwide is derived from edible vegetable oils such as soybean and canola oils (Gui et al., 2008) and this competes with the food and feeds supply industry, raising a heated debate on 'fuel vs food'. On the other hand, the cost of feedstocks accounts for 75-85% of total biodiesel production cost; thus, the price of biodiesel is generally higher than that of petrodiesel (Serra and Zilberman, 2013). This has been the major barrier to its commercialization on a large scale. Therefore, it is important

to develop non-edible and/or low-cost oil crops that meet certain requirements, including low agriculture inputs, high oil yield, and favorable fatty acid compositions, to increase the overall viability of biodiesel production.

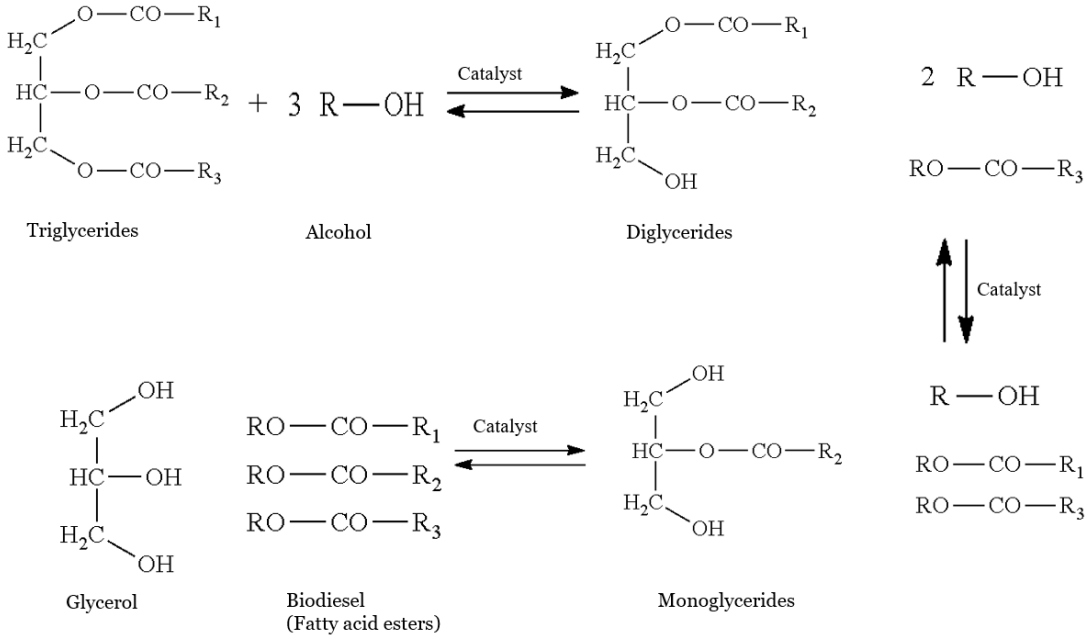


Fig. 1.1 The overall stoichiometric equation of transesterification in the presence of alcohol and catalyst.

Recent research has recognized camelina (*Camelina sativa* L. Crantz,), belonging to the *Brassicaceae* family and known as false flax or gold-of-pleasure, as a promising and sustainable oilseed crop for biodiesel production in North America (Krohn and Fripp, 2012; Urbaniak et al., 2008). Camelina seeds have a fairly high oil content (35-43% on a dry matter basis) (Gugel and Falk, 2006; Jiang et al., 2014). More importantly, it requires low cultivation inputs, has a short growing season, and is tolerant to drought, cool weather and insect pests (Iskandarov et al., 2014; Kirkhus et al., 2013). However, very limited research on the evaluation of the feasibility of camelina biodiesel production has been conducted even though the viability of *Camelina sativa* as an oil crop has been highly recognized.

1.2 Research Objectives

This project aims to evaluate the feasibility of biodiesel production from *Camelina sativa* oil through different aspects, including the property characterization of the parent oil and the resulting biodiesel, the optimization of production process, the purification of crude camelina biodiesel and the improvement of camelina biodiesel's oxidation stability. Specific objectives are as follows:

(1) To characterize the fatty acid profile of camelina oil and evaluate the fuel properties of camelina biodiesel against ASTM D6751 standard.
(2) To obtain the optimal transesterification reaction conditions for camelina biodiesel production.
(3) To investigate the effectiveness of fiber-based bio-absorbents (commercial BD Zorb, wood shavings and sawdust) on crude camelina biodiesel purification.
(4) To identify the most effective antioxidants and their suitable loading concentrations to improve camelina biodiesel's oxidation stability.

1.3 Thesis Organization

Chapter 2 presents a literature review on the production, purification and characterization of biodiesel as well as its oxidation stability. Chapter 3 provides an evaluation of biodiesel production from *Camelina sativa* grown in Nova Scotia, which mainly focuses on the fuel properties characterization of camelina biodiesel. Chapter 4 presents an optimization study on alkali-catalyzed camelina biodiesel production process by using a response surface methodology. An investigation on the purification efficiency of dry wash media in purifying crude camelina biodiesel was included in Chapter 5. Chapter 6 demonstrates the effects of synthetic antioxidants on the oxidative stability of camelina biodiesel. Chapter 7 provides the overall conclusions of this research and the recommendations for the future work.

CHAPTER 2: LITERATURE REVIEW

2.1 Camelina Oil Fatty Acid Profile

Camelina (*Camelina sativa* L. Crantz), a member of the *Brassicaceae* family, is an ancient crop originating from Northern Europe and Asia in 500 B.C., and relatively new to North America (Zubr, 1997). Camelina seeds contain about 35-43% of oil on a dry matter basis (Gugel and Falk, 2006). Camelina oil derived from camelina seeds naturally contains a high percentage of unsaturated fatty acids which is about 90 wt. %, specifically linolenic acid (C18:3; 32.6–38.2 wt.%), linoleic acid (C18:2;16.9–19.6 wt.%), oleic acid (C18:1; 14.5–19.7 wt.%) and gadoleic acid (C20:1; 12.4–16.2 wt.%) (Ciubota-Rosie et al., 2013a; Moser and Vaughn, 2010; Vollmann et al., 2007).

2.2 Fuel Properties of Camelina Biodiesel

The physical properties and chemical composition of biodiesel need to be comprehensively characterized as they would have a great impact on engine performances and pollutant formation, such as engine combustion process and greenhouse gas emissions (Szybist et al., 2007). A rigorous set of fuel specifications, including the ASTM D6751 in the North America and the EN 14214 in the European Union, were developed to identify the parameters that pure biodiesel (B100) must meet before being used as a pure fuel or being blended with petrodiesel.

The flash point is the lowest temperature at which biodiesel will form a flammable mixture with air. Flash point is an important parameter for assessing the overall flammability hazard of biodiesel during storage and shipping. Methanol content is critical for flash point because methanol is more flammable than biodiesel. High methanol content in biodiesel leads to a low flash point. Cloud point refers to the lowest temperature at which biodiesel starts having observable clusters of hydrocarbon crystals or starts becoming cloudy. Cloud point is a representative parameter of biodiesel flowability at low temperatures. Cetane number is a measure of biodiesel ignition performance inside engine by comparing with a reference fuel (pure C16:0). Cetane number represents how quickly the biodiesel starts to burn during the combustion of biodiesel. Acid number is a parameter

which indicates the acidity level of biodiesel and is important as a high acid number may cause damage to rubber lines and other engine elements.

The biodiesel derived from camelina must meet these specifications before being used in diesel engines. However, very limited research has been conducted to characterize camelina biodiesel properties comprehensively. Ciubota-Rosie et al., (2013) reported that camelina biodiesel exhibited poor oxidative stability (1.3 hours) and low cetane number (42.8) and that both did not comply with ASTM D6751; however, other properties of camelina biodiesel were in good agreement with ASTM D6751, such as low-temperature operability (cloud point and cold soak filtration test), viscosity, distillation properties and little carbon residue and sulfur content, etc. However, Moser and Vaughn, (2010) demonstrated that cetane number (52.8) of camelina biodiesel met ASTM D6751 minimum specification (47), but its oxidative stability was not satisfactory.

2.3 Optimization of Transesterification Reaction Conditions

Vegetable oils are naturally occurring triglycerides (TAGs) that can be used to produce biodiesel via alcoholysis in the presence of a catalyst through a process called transesterification. Methanol is the most widely used alcohol for biodiesel production. The main by-product of this chemical reaction is glycerol. The transesterification of parent oil generates fatty acid methyl esters (FAME) that theoretically possess the fatty acid profile of the parent oil. Hence, biodiesel derived from camelina oil is a mixture of 10-12%, 37-40%, and 48-50% saturated, monounsaturated and polyunsaturated methyl esters respectively based on the fatty acid profile of camelina oil (Soriano Jr and Narani, 2011). There are two ways to express the yield of biodiesel obtained from the transesterification process: product yield and FAME yield. Product yield [Eq. (1)] indicates the amount of biodiesel would be produced from raw oil. FAME yield [Eq. (2)] is determined by the amount of FAME in the resulting biodiesel with respect to feedstock oil, which is an indicator of the quality of biodiesel.

$$\text{Product yield} = \frac{\text{mass of biodiesel}}{\text{mass of oil}} \times 100\ \% \quad (1)$$

$$\text{FAME yield} = \frac{\text{mass of FAME}}{\text{mass of biodiesel}} \times 100\ \% \quad (2)$$

In the transesterification process, the quantity and quality of the resulting biodiesel are also influenced by some reaction variables, including the reaction temperature, reaction time, molar ratio of methanol/oil, and catalyst concentration. These processing parameters need to be optimized to maximize the yield and quality. The application of a catalyst during transesterification plays a significant role in reaction efficiency. Many researchers have employed different kinds of catalysts to enhance transesterification, such as acid, base and enzyme. Base catalysts have been recognized to be the most suitable catalysts in an industrial-scale biodiesel production due to their low cost, low reaction temperature and relatively short reaction time required compared to acid and enzyme catalysts (Atadashi et al., 2013). For the base catalyst, potassium hydroxide (KOH) and sodium hydroxide (NaOH) are most typically used for biodiesel production. The addition of excess methanol can also enhance the transesterification process to achieve a high FAME yield (Rashid and Anwar, 2008). Reaction temperature and time are also critical to biodiesel production but vary with different feedstocks and the amount of oil applied. The maximum product yield and FAME yield would be obtained via an optimization study of transesterification reaction conditions. For instance, Wu and Leung, (2011) has shown that the maximum camelina biodiesel product yield (95.8%) and FAME yield (98.4%) were achieved under the optimal reaction conditions, namely 8:1 methanol to oil molar ratio, 1 wt.% catalyst (potassium hydroxide, KOH) concentration, 70 minutes reaction time and 50 ºC reaction temperature). Although there have been a few studies related to camelina biodiesel production, the optimization of transesterification reaction conditions has not been thoroughly investigated.

2.4 Camelina Biodiesel Purification

A downstream purification process after conversion of parent oils to the crude biodiesel is required to ensure the biodiesel quality meeting the ASTM specifications. The crude camelina biodiesel produced from transesterification contains a number of impurities, such as alcohol, catalyst, water, glycerol, unreacted mono-, di- and triglycerides, free fatty acids and soap (Atadashi et al., 2011; Stojković et al., 2014). Glycerol is the by product and majority of it can be removed from biodiesel by phase separation because of the density and polarity difference between them, but trace amounts of glycerol could remain in

biodiesel after separation. ASTM D6751 specifies total glycerol in biodiesel should be lower than 0.24 wt. %. Exceeding this limit will cause undesirable deposits which may clog fuel lines and pumps. Naturally occurring free fatty acids (FFA) in vegetable oil can react with catalyst like KOH or NaOH to form soap, and this is called saponification which happens in parallel with transesterification. Similar to glycerol, a high soap content can result in engine wear problems and negatively affect the engine's lifespan (Atadashi et al., 2011; Stojković et al., 2014). Although there is no explicit specification of soap content in the ASTM D6751, the generally acceptable soap limit should be lower than 66 ppm in the case that KOH is used as a catalyst in the biodiesel production process. Ash could be generated while burning potassium or sodium enriched biodiesel in diesel engine cylinder. FFA percentage in biodiesel is closely related to its acid number (AN; ≤0.5 mg KOH/g), and a high level of FFA can cause corrosion of the diesel engine components. The presence of excessive water content (WC; ≤500 ppm at ASTM D6751) in biodiesel might reduce the heat efficiency of fuel combustion, enhance the hydrolysis of FAME and even form ice crystals resulting in gelling of fuels while at low-temperature conditions (Atadashi, 2015; Stojković et al., 2014). Therefore, proper purification processes are essential to decrease or minimize glycerol, methanol and soap contents in the crude biodiesel to fully meet the biodiesel specifications.

In general, there are two ways to purify crude biodiesel: water washing and dry washing. Water washing is the traditional way used to remove water-soluble impurities in crude biodiesel. Although water washing enables crude biodiesel to be purified efficiently, this wet washing purification process generates an enormous amount of wastewater and aqueous effluent that have a detrimental impact on the environment. Therefore, many efforts have been made to develop an alternative purification process, dry washing. Ion exchange resin is one type of dry wash media that has been intensively investigated for biodiesel purification. Wall et al., (2011) studied the performance of commercially available resins on soap and glycerol removal and have stated that four mechanisms in the dry wash purification *via* resin were involved, including physical filtration, adsorption, ion exchange and glycerol/soap interaction. Filtration is a physical action that can filter out insoluble impurities from the crude biodiesel and adsorption can remove soluble impurities *via* chemical action due to the polar pores and surface of adsorbent particles. Ion exchange

involves the chemical breakdown of the impurities, such as the sodium or potassium ions in soap, which can be replaced by hydrogen ion from resin and form FFA. Glycerol/soap interaction is based on the high affinity between them.

Recently, some fiber-based dry wash media from biomass waste streams, such as spent tea waste (Fadhil et al., 2012), rice husk ash (Manique et al., 2012) and eucalyptus pulp (Squissato et al., 2015), have demonstrated satisfactory efficiency for the removal of crude biodiesel impurities. BD Zorb, one of the commonly used fiber-based absorbents, consists of premium hardwood shavings that are specially dried and chemically treated. However, very limited research has been conducted to investigate the effectiveness of BD Zorb or other wood materials on the removal of impurities from crude biodiesel.

2.5 Improvement of Camelina Biodiesel Oxidative Stability

2.5.1 Factors That Affect Oxidative Stability

The poor oxidative stability of camelina biodiesel has been illustrated by a few studies, and different factors could influence the biodiesel oxidative stability. The degree of unsaturation of raw oil fatty acid composition is the most significant factor for biodiesel oxidative stability (Zuleta et al., 2012). Other factors that may impact biodiesel oxidative stability during storage time include ambient air, light, elevated temperature, a trace amount of catalytic metals, storage container materials (external contaminants) and peroxides (Knothe, 2007). Air exposure is the major factor that results in biodiesel autoxidation during the storage time; reducing the contact area between air and biodiesel can minimize biodiesel autoxidation (Knothe, 2006).

2.5.2 Oxidative Stability Monitoring

Autoxidation is characterized by oxidative stability index (OSI) or induction period (IP). The OSI or IP of biodiesel can be measured by Rancimat method, which is the standard testing method in EN 14112. OSI is the period of time between passing air through samples to the appearance of secondary oxidation products (volatile organic acids). So a higher OSI represents a better oxidative stability. Adding antioxidants to lipid or biofuels can delay the oxidation initiation, therefore, increasing the induction period until the antioxidant is exhausted (Bondioli et al., 1995; Dunn, 2008).

In addition to OSI, peroxide value (PV) is also commonly used to evaluate the amount of primary oxidation products (such as hydroperoxides) in the edible vegetable oil industry by titration. Although PV provides information concerning the extent of oil oxidation, it might not be suitable for monitoring oil or biodiesel storage stability over a long period of time. This is because PV tends to increase at the early stage of oxidation and then decreases as hydroperoxides decompose to form secondary oxidation products (Dunn, 2002).

2.5.3 Adding Antioxidants to Improve Oxidative Stability

Typically, biodiesel produced from vegetable oils needs to be treated with a suitable amount of antioxidants to meet the oxidative stability requirement in biodiesel standards, EN14214 or ASTM D6751, that of 8 hours and 3 hours respectively. This is due to the inherent nature of vegetable oil, containing a relatively high percentage of unsaturated fatty acids. Antioxidants that occur naturally in vegetable oils, such as vitamin E (tocopherols and tocotrienols), are called natural antioxidants. The natural antioxidants level in biodiesel can be influenced by the refining process of vegetable oil before the transesterification process (Fernández et al., 2010). There are also different kinds of synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), *tert*-butylhydrooquinone (TBHQ) and propyl gallate (PrG). Those synthetic antioxidants can be deliberately added into biodiesel to improve biodiesel oxidative stability. However, the effectiveness of those antioxidants varies. On the other hand, the loading concentration of antioxidants also plays an important role in their effectiveness. Research from Dunn, (2005) showed that PrG, BHT and BHA were the most effective antioxidants for soybean methyl ester, and α-Tocopherol was the least effective one. This study suggested that up to 3000 ppm loading concentration of BHA could effectively minimize auto-oxidation during long-term storage. 50 ppm BHT and 50 ppm TBHQ were added into the same amount distilled palm oil methyl ester, the oxidative stability index (OSI) for BHT and TBHQ were 6.42 hours and 8.85 hours respectively (Liang et al., 2006). Therefore, the type of antioxidant and the loading concentration of antioxidant are two important factors that can impact biodiesel oxidative stability.

CHAPTER 3: AN EVALUATION OF BIODIESEL PRODUCTION FROM *CAMELINA SATIVA* GROWN IN NOVA SCOTIA

Contribution statement:

I was responsible for raw materials collection, biodiesel preparation, experiment design and conduction, data analysis and manuscript preparation.

3.1 Abstract

Camelina sativa has recently attracted great interest as an energy crop for biodiesel production in North America. To assess the feasibility of biodiesel production from camelina, the cultivation conditions, camelina oil fatty acid profile, alkali-catalyzed transesterification process and fuel properties of camelina biodiesel were investigated. Unrefined camelina oil, containing 10%, 33.2% and 56.8% of saturated, monounsaturated and polyunsaturated fatty acids respectively, was used to synthesize biodiesel. The conversion rate of *Camelina sativa* oil to its methyl esters was 96% under optimal reaction conditions. Most fuel properties of the resulting camelina biodiesel were in good agreement with specifications of the American Society for Testing and Materials (ASTM D6751) and European standard (EN 14214), such as kinematic viscosity, acid number, flash point, sulfur content, total glycerol content. Its cetane number (49.7) was satisfactory according to ASTM D6751, but not for EN 14214. Camelina biodiesel exhibited poor oxidative stability (1.9 h) resulting from the high percentage of polyunsaturated fatty acid methyl esters.

3.2 Introduction

In the context of biodiesel production, the availability of parent oils greatly depends on geographical regions. Large amounts of biodiesel are currently derived from rapeseed oil in European countries, soybean oil in the United State and canola oil in Canada (Gui et al.,

2008). It is therefore essential to identify plant species that are either suitable for local cultivation conditions or broadly adapted to increase their economic viability. On the other hand, in response to the "Fuel vs Food" issue caused by using edible crops for biofuels production, researchers and industrial practitioners are actively seeking and/or developing dedicated energy crops that are less competitive with traditional crops in terms of water, land and nutrient requirements. There are a few successful practices, for instance, biodiesel production from non-edible *Jatropha curcas* oil in South Asia (Meher et al., 2013; Mofijur et al., 2012), and baobab (*Adansonia digitata*) oil in South Africa (Modiba et al., 2014). However, these species cannot be readily grown in Canada due to poor climatic adaptation.

Recent research has recognized *Camelina sativa*, belonging to *Brassicaceae* family, as a promising and sustainable oilseed crop for biodiesel production in North America (Krohn and Fripp, 2012; Urbaniak et al., 2008). Camelina seed has a high oil content (35–43% on a dry matter basis) (Gugel and Falk, 2006; Jiang et al., 2014) and camelina oil naturally contains a high percentage of unsaturated fatty acid compositions (about 90%) (Moser and Vaughn, 2010; Vollmann et al., 2007). The fatty acid profile of camelina oil varies with different cultivation conditions such as genotype, location, environmental conditions and fertilizer inputs (Jiang et al., 2014; Zubr, 2003). Camelina has a short growing season, and is tolerant to drought, cool weather and insect pests (Iskandarov et al., 2014; Kirkhus et al., 2013), thus it becomes a desirable and low-cost feedstock for biodiesel production. Moreover, Krohn and Fripp, (2012) stated that camelina biodiesel is more environmentally viable than traditional soybean and canola biodiesel due to its lower life cycle energy requirement and low greenhouse gas emissions when taking land use change into consideration.

As mentioned in Section 2.2, a rigorous set of fuel specifications (ASTM D6751 and the EN 14214) were developed to identify the parameters that pure biodiesel (B100) must meet before being used in diesel engines. Some of the specified values in the standards are closely related to the transesterification and purification processes, such as FAME content, total glycerol and free glycerol contents, methanol content and water content. Alternatively, the kinematic viscosity, cold flow properties, cetane number, distillation temperature and oxidative stability depend primarily on the fatty acid composition of the parent oil (Ciubota-Rosie et al., 2013). The chain length of fatty acid esters and the degree

of unsaturation reveal critical effects on the biodiesel properties (Knothe, 2005). Hence, it is important to determine the fatty acid profile of the parent oil proposed for biodiesel production in order to achieve satisfactory biodiesel properties that meet the specifications identified by the standards ASTM D6751 or EN 14214.

This chapter examines the feasibility of camelina grown in Nova Scotia for biodiesel production. The fatty acid profile of camelina oil was characterized and compared to that of camelina grown in other regions. Alkali-catalyzed transesterification was applied to convert camelina oil into biodiesel, and the effects of reaction parameters (temperature, reaction time, molar ratio of methanol to oil and catalyst concentration) on the biodiesel yield were assessed. The fuel properties of the resulting biodiesel were thoroughly evaluated according to the testing methods specified in the ASTM D6751 or EN 14214.

3.3 Materials and Methods

3.3.1 Materials

Camelina oil was cold pressed from *C. sativa* L. Crantz CDI007 seeds grown in Canning, Nova Scotia, Canada. Potassium hydroxide (>85%) in the form of pellets, analytical grade methanol (>99%), calcium chloride anhydrous and hexane (>99%) were purchased from Fisher Scientific Ltd., Canada. Sodium methoxide (25 wt. % solution in methanol) and the standard reference solution (GLC 96) was purchased from Sigma–Aldrich, Canada and Nu-Chek Prep. Inc., USA respectively.

3.3.2 Identification of *Camelina sativa* Oil Fatty Acid Profile

Camelina oil was methylated according to ISO 5509 standard (Animal and vegetable fats and oils — Preparation of methyl esters of fatty acids). The prepared sample was injected into an Agilent 7890A GC equipped with a Flame Ionization Detector (FID) at 260 °C and an Agilent DB-23 column (50%- cyanopropyl-methylpolysiloxane; 30 m length × 0.25 mm internal diameter × 0.25 m thickness; high polarity). The carrier gas was helium, and the oven temperature was initially set at 190 °C then was increased to 250 °C at a heating of rate of 40 ◦C/min and was maintained at 250 °C for 3.5 min. The fatty acid methyl esters were identified by comparing their specific retention times to those of a standard reference solution (>99%) of GLC 96 (Nu-Chek Prep. Inc). The moisture and

volatiles, free fatty acid, insoluble impurities, unsaponifiable matter were determined according to AOCS Ca 2c-25, AOCS Ca 3a-46, AOCS Ca 6a-40 and AOCS Ca 5a-40 respectively. The phosphorus content of camelina oil was determined in accordance with AOAC 984.27.

3.3.3 Biodiesel Synthesis

Cold pressed camelina oil was used to produce camelina biodiesel at a laboratory scale via an alkaline-catalyzed transesterification process. A typical camelina biodiesel synthesis was as follows: 50 g of camelina oil was added to a 300-mL flask and placed in a water bath at a set temperature. A pre-calculated amount of methanol solution containing completely dissolved KOH was added to the camelina oil. The reaction was carried out with a constant 300 rpm agitation rate and stopped once the preset time was reached. The reaction mixture was transferred to a separatory funnel and allowed to stand for 30 min for phase separation, and then the glycerol layer under the crude biodiesel was drawn off. The crude biodiesel remaining in the separatory funnel was washed by a few batches of distilled water until the water layer became completely translucent. Camelina biodiesel (after the water washing) was dried by adding calcium chloride and then centrifuged to remove the water saturated calcium chloride, giving purified biodiesel for further analysis.

3.3.4 Characterization of Biodiesel Properties

Two liters of the prepared camelina biodiesel were submitted to a certified lab, Alberta Innovates-Technology Futures (Edmonton, Alberta) for testing the fuel properties against the ASTM D6571 and EN14214, including the density at 15 °C (ASTM D4052), cloud point (ASTM D5773), cetane number (ASTM D613), cold soak filterability test (ASTM D7501), flash point (ASTM D93), carbon residue (ASTM D4530), total sulfur (ASTM D5453), particulate contaminant (ASTM D7321), free glycerol, total glycerol, monoglyceride, diglyceride and triglyceride (ASTM D6584), methanol content (EN 14110), methyl ester content (EN 14103), metal I (Na + K), metal II (Ca + Mg), and phosphorus content (modified EN 14538). The kinematic viscosity at 40 °C (ASTM D445), water content (EN ISO 12937), acid number (EN 14104), oxidative stability (EN 14112)

were tested in the Department Engineering, Dalhousie Faculty of Agriculture, Nova Scotia, Canada.

3.4 Results and Discussion

3.4.1 Cultivation

Camelina was grown at two locations in Nova Scotia, Truro (lat. 45.36 ◦N, long. 63.28 ◦W) and Canning (lat. 45.16 ◦N, long. 64.43 ◦W). Camelina can be seeded as early as soil conditions permit and the recommended seeding rate ranged from 5 to 7 kg ha^{-1} (4.5–6.2 lb ac^{-1}) or 500–700 seeds m^{-2}. The row spacing of 15 cm (6 inches) and seed depth of 0.5 cm (1/4 inch) were recommended. Nitrogen application was recommended at 100–125 kg ha^{-1}, and the splitting nitrogen application as ½ at seeding and ½ at early flowering was preferred to maximize the seed yield, approximately 1500 kg ha^{-1} in average. Camelina seed oil content and protein content ranged from 30 to 40% and 25–30%, respectively. The oil yield of camelina can be expected from 450 to 600 kg ha^{-1}. It has been demonstrated that an increase in nitrogen application rate increased the seed yield, however, it also decreased the oil content in camelina seeds which was the desirable component for biodiesel production (Jiang et al., 2014). Two products for weed control have been registered. Quizalofop (Assure II; Yuma GL) herbicide was used for grassy weed control and glyphosate for pre-harvest weed control. Although not registered, Treflan and Bonanza have been applied safely as pre-plant incorporated herbicides on camelina for broadleaf and grassy weed control. The most common plant disease observed in Nova Scotia on camelina was downy mildew (*Peronospora parasitica*) (Séguin-Swartz et al., 2009). Sclerotinia (*Sclerotinia sclerotiorum*), which had not been observed previously, started to show in the field after two years. There is currently no registered fungicide for disease control on camelina.

3.4.2 Characterization and Comparison of Camelina Oil Fatty Acid Profile

The camelina oil fatty acid profile identified by GC is depicted in Fig. 3.1. The fatty acid composition of camelina oil is listed and compared to the published values as shown in Table 3.1. The linolenic acid (C18:3, 33.5 wt. %), is the primary fatty acid, followed by 14.4 wt.% of oleic acid (C18:1), 19.1 wt.% of linoleic acid (C18:2), 15.0 wt.% gadoleic

acid (C20:1), 5.5 wt.% of palmitic acid (C16:0), 2.4 wt.% of stearic acid (C18:0), 3.1 wt.%. of erucic acid (C22:1) and other fatty acids with trace amounts. Based on the degree of saturation, camelina oil is comprised of 10% saturated, 33.2% monounsaturated, and 56.8% polyunsaturated fatty acids. This is consistent with results reported in the previous studies (Wu and Leung, 2011; Moser and Vaughn, 2010; Hrastar et al., 2009; Fröhlich and Rice, 2005). In addition to the fatty acid profile, moisture and volatiles, free fatty acid, insoluble impurities, unsaponifiable matters and phosphorous content of camelina oil were tested and listed in Table 3.2.

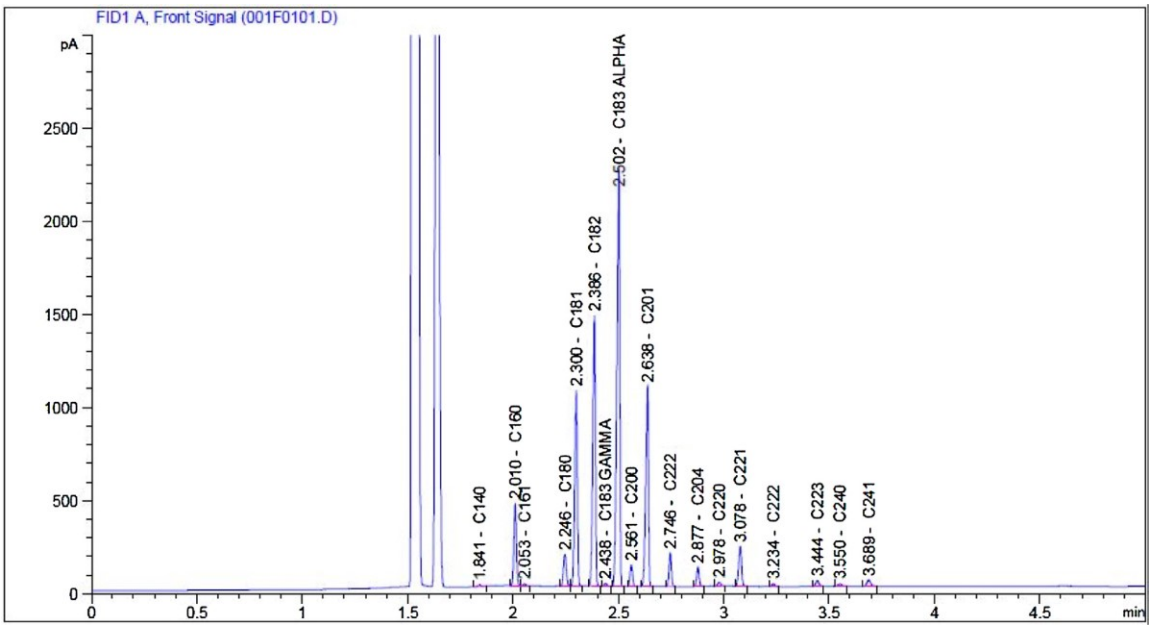


Fig. 3.1 The gas chromatogram of *Camelina savita* oil fatty acid composition.

Table 3.2 The properties of *Camelina sativa* oil used in this study.

Properties	Content (%)
Moisture and volatiles	0.01
Insoluble impurities	0.05
Unsaponifiable matter	0.7
Phosphorus	<20 ppm
Free fatty acid	0.67

3.4.3 Synthesis of Biodiesel

Biodiesel was synthesized using a transesterification process as described in Section 3.3.3. The effects of reaction time, temperature, catalyst loading and molar ratio of methanol to oil were investigated. The influence of the reaction time on the camelina biodiesel conversion rate is shown in Fig. 3.2a. The highest conversion rate (96.1%) was achieved at 33 min when the temperature, amount of catalyst and molar ratio of methanol/oil were set at 40 °C, 1.25 wt. % and 8:1 respectively. An extended reaction time did not further increase the conversion rate. Fig. 3.2b presents the effect of reaction temperature (range from 30 to 50 ◦C) on the biodiesel conversion rate. It was observed that the conversion rate decreased along with an increase in temperature beyond 40 ◦C, where the maximum conversion rate was obtained. This could result from the evaporation of methanol under a relatively high temperature.

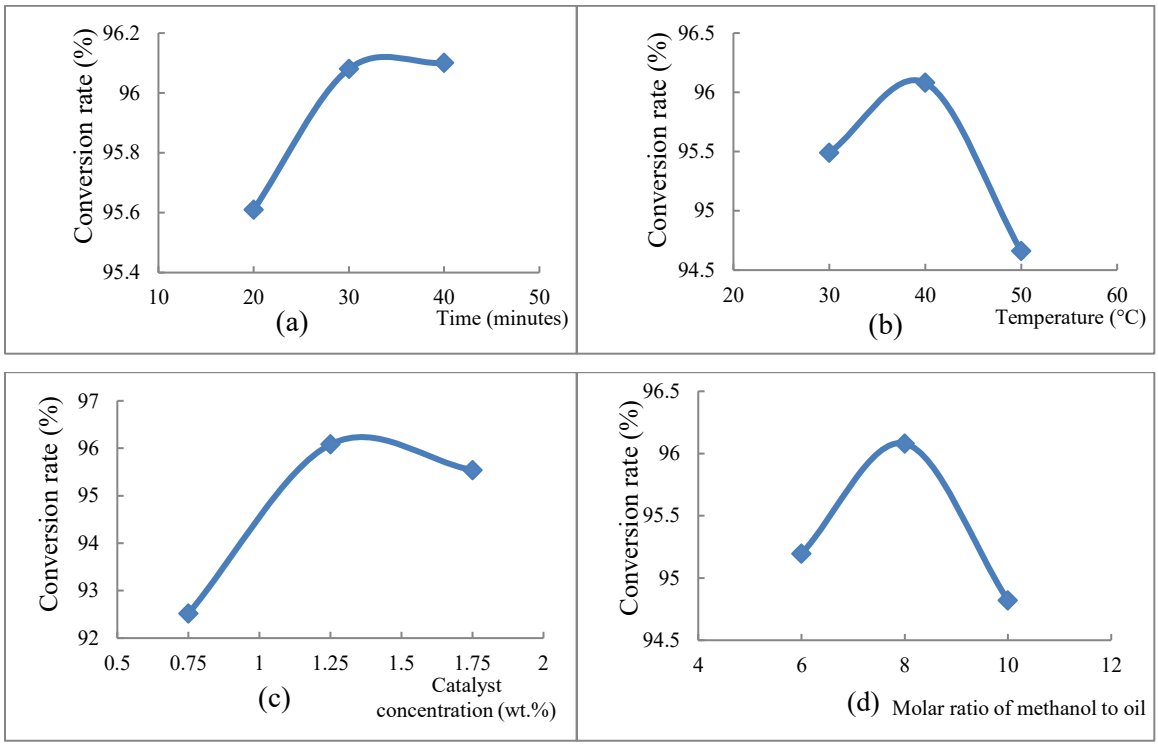


Fig. 3.2 The effect of (a) reaction time (min), (b) reaction temperature (°C), (c) catalyst concentration (wt. %) and (d) molar ratio of methanol to oil on the conversation rate (%).

Table 3.1 The fatty acid composition (wt.%) of *Camelina sativa* oil in this study compared to other published values.

Fatty acid	C: D	Present study	Ciubota-Rosie et al. (2013)	Moser and Vaughn, (2010)	Wu and Leung, (2011)	Fröhlich and Rice, (2005)	Hrastar et al., (2009)
Myristic acid	C14:0	0.1	0.05	0.1	0.2	-	-
Palmitic acid	C16:0	5.5	5.16	6.8	5.1	5.4	5.7
Palmitoleic acid	C16:1	0.1	0.04	-	0.3	-	-
Stearic acid	C18:0	2.4	2.68	2.7	2.4	2.6	3.37
Oleic acid	C18:1	14.4	15.21	18.6	17.6	14.3	15.01
Linoleic acid	C18:2	19.1	17.9	19.6	18.7	14.3	18.48
Linolenic acid	C18:3	33.5	34.64	32.6	28.6	38.4	34.72
Arachidic acid	C20:0	1.5	1.44	1.2	1.8	0.25	1.83
Gadoleic acid	C20:1	15	15.14	12.4	11.9	16.8	12.71
Eicosadienoic acid	C20:2	2.2	2.17	1.3	1.9	-	1.48
Eicosatrienoic acid	C20:3	-	-	0.8	-	-	1.05
Arachidonic acid	C20:4	1.4	1.47	-	-	-	-
Behenic acid	C22:0	0.3	0.3	-	0.8	1.4	0.37
Erucic acid	C22:1	3.1	2.57	2.3	4.2	2.9	3.24
Clupanodinic acid	C22:2	0.2	-	-	0.4	-	-
Docosatrienoic acid	C22:3	0.4					
Docosahexaenoic acid	C22:6	-	0.62	-	-	-	-
Lignoceric acid	C24:0	0.2	0.14	-	-	-	-
Nervonic acid	C24:1	0.6	-	-	-	-	0.64

Notes: C: D denotes the number of carbons and the number of double bonds in each fatty acid.

The effect of the catalyst loading was examined in a catalyst concentration range of 0.75–1.75 wt. % when the reaction temperature, molar ratio of methanol/oil and reaction time were set at 40 ◦C, 8:1 and 40 min. The results are illustrated in Fig. 3.2c. The conversion rate was significantly increased to 96.2% from 92.6% when the catalyst loading was increased from 0.75 wt. % to 1.3 wt. %. However, a further increase in catalyst concentration over 1.3 wt.%, favored the formation of soap and thus decreased the conversion rate. As seen in Fig. 3.2d, the molar ratio of methanol/oil also influenced the conversion process. The maximum conversion rate was 96.3% at 8:1 of the molar ratio of methanol/oil. The conversion rate decreased when the molar ratio of methanol/oil was further increased. This observation is attributed to the appearance of an emulsion. Wu and Leung, (2011) has reported a similar trend of the camelina biodiesel conversion rate, affected by these four independent variables. In this study, a high conversion rate (96%) has been achieved at the optimal reaction conditions.

3.4.4 Evaluation of the Fuel Properties of Camelina Biodiesel

The properties of the resulting camelina biodiesel in this study are summarized in Table 3.3 together with the standards specified in the ASTM D6751 and EN 14214 as well as the published values in literature. The EN 14214 standards indicate a satisfactory FAME content to be greater than 96.5 wt.%, while this value is not specified in the ASTM D6751. The FAME content obtained in this study was 98.5%, complying with the EN14214 standard. The methanol content (<0.01 vol.%) met the requirements in both standards. The flash point of 152 °C was much higher than of 96 °C as defined in standards, implying that camelina biodiesel is safe to be handled during the process of transport and storage. The density, kinematic viscosity, water content, acid number, total glycerol, free glycerol, monoglyceride, diglyceride and triglyceride contents were tested and all of them adhere to the specifications in ASTM D6751 and EN 14214 standards. Camelina biodiesel also inherently contained negligible amounts of undesirable elements and compounds such as sulfur (S), phosphorus (P), sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg). In accordance with the required testing methods, the trace amounts of all these components were determined and found to be lower than the limits defined in both ASTM D6751 and EN 14214 standards. These properties are also comparable to the reported data

in other research (Ciubota-Rosie et al., 2013; Soriano Jr and Narani, 2011; Moser and Vaughn, 2010; Fröhlich and Rice, 2005).

The cold flow properties of biodiesel are extremely critical in assessing the quality of biodiesel. Poor cold flowability is a common concern in the biodiesel industry as biodiesel is susceptible to start-up and low-temperature operability problems under cold weather conditions. This is mainly caused by the formation of crystals and solid precipitates at low temperatures, which could clog the vehicle fuel lines and filters (Soriano Jr and Narani, 2011). It has also been documented that even a trace amount of minor constituents can impact biodiesel's low-temperature performance (Dunn, 2009). These minor constituents, often referred to contaminants, may originate from parent vegetable oils or be introduced during the transesterification, purification and even storage processes.

Cloud point (CP) is one of the representative parameters, typically used to evaluate the cold flow properties of biodiesel. Cloud point is defined as the temperature at which biodiesel starts forming cloudy suspensions and visible crystals. Generally, a relatively high CP value indicates a poor cold flowability. In the present research, the CP value of camelina biodiesel was determined to be −1.6 ◦C, lower than the reported CPs of camelina biodiesel in other studies: 0 °C (Ciubota-Rosie et al., 2013), 2.7 °C (Soriano Jr and Narani, 2011) and 3 °C (Moser and Vaughn, 2010; Fröhlich and Rice, 2005) . The CP of biodiesel is closely related to the melting point of fatty acid esters. The lower melting point is, the harder for biodiesel to form crystals at cold weather. Knothe, (2005) has demonstrated that the melting point of fatty acid esters was decreased with an increase in the degree of unsaturation. Camelina biodiesel inherently contains a high percentage of unsaturated fatty acid esters, so it is not surprising that the CP was relatively low (−1.6 ◦C). Although the value of CP is a good representative for biodiesel cold flow properties, it cannot adequately assess the low-temperature operability or performance of the biodiesel in vehicle tanks and fuel systems (Westbrook and LeCren, 2003). Another more useful measure is to conduct a cold soak filtration test (CSFT), identifying the potential of biodiesel to clog fuel lines and filters. CSFT yields a period of time (in seconds) that is consumed by passing 300 mL of biodiesel through a 0.7 um glass fiber filter under 71–85 KPa vacuum.

Table 3.3 The properties of *Camelina sativa* biodiesel evaluated in this study accompanied with data reported in the literature.

Properties	Units	Present study	ASTM D6751-09	EN 14214-10	Ciubota-Rosie et al. (2013)	Soriano Jr and Narani, (2012)	Moser and Vaughn, (2010)	Fröhlich and Rice, (2005)
Density at 15 °C	kg/m^3	887.6	-	860-900	888	-	-	882
Kinematic viscosity at 40 °C	mm^2/s	3.9	1.9-6	3.5-5	4.3	4.32	4.15	6.43
Cetane number	-	49.7	≥ 47	≥ 51	42.76	-	52.8	-
Acid number	mg KOH/g	0.25	≤ 0.5	≤ 0.5	0.15	-	0.31	0.33
Iodine value	g I2/g	-	-	≤ 120	152	-	151	153
Water content	mg/kg	427	-	≤ 500	120	-	-	-
Methanol content	vol.%	<0.01	≤ 0.2	≤ 0.2	0.0121	-	-	≤ 0.1
Flash point	°C	152	≥ 93	≥ 101	152	172	-	-
Cloud point	°C	-1.6		-	0	2.7	3	3
Cold soak filterability test	s	477	≤ 360	-	246	-	-	-
Cold filter plugging point	°C	-	-	-	-4	1.1	-3	-3
Oxidative stability, 110 °C	hour	1.9	≥ 3	≥ 6	1.3	0.6	2.5	-
Vacuum distillation end point[a]	°C	-	≤ 360	-	369	369	-	-
Copper strip corrosion (3h, 50 °C)	Classification	-	≤ 3	1	1A	-	-	-
Carbon residue	wt.%	0.003	≤ 0.05[b]	≤ 0.3[c]	0.019	-	-	-
Sulphur content	mg/kg	3.6	≤ 15	≤ 10	0.57	5.46	3	-

Properties	Units	Present study	ASTM D6751-09	EN 14214-10	Ciubota-Rosie et al. (2013)	Soriano Jr and Narani, (2012)	Moser and Vaughn, (2010)	Fröhlich and Rice, (2005)
Phosphorus content	mg/kg	<2.0	≤ 10	≤ 4.0	<0.1	-	0	-
Na and K	mg/kg	<1.0	≤ 5.0	≤ 5.0	0.11	-	-	-
Ca and Mg	mg/kg	0.31	≤ 5.0	≤ 5.0	0.16	-	-	-
Sulphated ash content	wt.%	-	≤ 0.02	≤ 0.02	0.0013	-	-	0.01
Water & sediment	vol.%	-	<0.2	-	0	-	-	-
Total contamination	mg/kg	-	-	≤ 24	7.3	-	-	-
Methyl ester content	wt.%	98.5	-	≥ 96.5	97.5	-	-	98.4
Linolenic acid methyl ester	wt.%	32.7	-	≤ 12.0	34.2	-	-	38.4
Polyunsaturated (≥ 4 double bond) methyl ester	wt.%	-	-	≤ 1	2.08	-	-	-
Monoglyceride content	wt.%	0.199	-	≤ 0.8	0.579	-	-	-
Diglyceride content	wt.%	<0.09	-	≤ 0.2	0.171	-	-	-
Triglyceride content	wt.%	<0.001	-	≤ 0.2	0.107	-	-	-
Free glycerol	wt.%	<0.001	≤ 0.02	≤ 0.02	0.006	-	-	≤ 0.1
Total glycerol	wt.%	0.055	≤ 0.24	≤ 0.25	0.189	-	-	≤ 0.18

Notes: ASTM = American Society for Testing and Materials; EN= European standard
a: atmospheric equivalent temperature 90% recovery
b: on 100% sample
c: on 10% distillation residue

In this study, the CSFT time was determined to be 477s, apparently longer than the standard (360s) indicated in ASTM D6751. However, as shown in Table 3.3, the CSFT time of 246s reported by Ciubota-Rosie et al. (2013) fully conformed to the ASTM standard. A long CSFT time is generally attributed to the presence of saturated monoglyceride (MAG) and free steryl glucosides (FStG) which have relatively high melting points (77 °C for monopalmitin, 82 °C for monostearin and 240 °C for sitosteryl glycoside) and easily crystallize when temperature decreases (Lee et al., 2007; Van Gerpen et al., 1997). The MAG content in the present study was 0.199 wt.%, which was much lower than the specified value (≤0.8 wt.%) in EN 14214, thus, the influence of saturated MAG on CSFT was very limited in this case. Naturally occurring acylated steryl glucoside in vegetable oils can be hydrolyzed during transesterification process and then form FStG (Dunn, 2009). The concentration of FStG in biodiesel is highly associated with the refining process of the respective parent oils. Hoed et al., (2008) have demonstrated that biodiesel derived from the crude soybean oil had much higher FStG concentration (272 ppm) than that in biodiesel made from refined-bleached soybean oil (64 ppm). In this study, the camelina oil used was cold pressed without any refining processes, while Ciubota- Rosie et al. (2013) used the refined camelina oil. This could well explain the observations, a longer CSFT time of 477s in our study while a relative low CSFT time of 246s in the study of Ciubota-Rosie et al. (2013).

Cetane number (CN) is also an important parameter for evaluating the quality of biodiesel. Conceptually similar to the octane number for gasoline, it is the prime indicator of biodiesel ignition performance inside a combustion cylinder (Knothe, 2005). In particular, it is a measure of the fuel's ignition delay, defined as a time period between injection of the fuel into the cylinder and the first identifiable pressure increase during the fuel combustion. Thus, the cetane number basically represents how quickly the fuel starts ignition during combustion in a diesel engine cylinder. A higher biodiesel cetane number indicates a quicker ignition time. A low biodiesel CN can lead to an incomplete combustion, which may cause engine knocking and an increase in NOx emissions (Monyem and Van Gerpen, 2001). The minimum biodiesel CN specified in the ASTM D6751 and EN 14214 is 47 and 51 respectively, while the minimum CN of 40 for the conventional diesel fuel prescribed in ASTM D975 is relatively low compared to that of

biodiesel. This is because the conventional diesel fuel contains much more aromatic compounds (naturally low CN) than biodiesel. The CN of biodiesel mainly depends on the chain length and the degree of unsaturation of fatty acid esters (Knothe, 2005). Ramírez-Verduzco et al., (2012) have demonstrated that biodiesel CN increased with an increasing chain length and decreased with an increase in the number of the double bonds. Although Ciubota-Rosie et al. (2013) reported in their study that the CN of camelina biodiesel was 42.76, not meeting the limit of 47 in the ASTM D 6751 nor the limit of 51 in the EN14214 (Ciubota-Rosie et al., 2013), in this study, the CN of camelina biodiesel was determined to be 49.7, which complied with the ASTM D6751 standard while it was still not satisfactory for the EN14214 standard. Other researchers reported that the cetane number of camelina biodiesel was 52.8, which fully met both ASTM D6751 and EN 14214 standards (Moser and Vaughn, 2010). The variations of CN values among these three studies could be attributed to the slight difference in the fatty acid profile of their respective parent camelina oils grown in different regions and cultivation conditions.

Oxidative stability of biodiesel is another important measure of the quality of biodiesel. Poor stability will lead to an increased kinematic viscosity during the storage time, due to the generation of relatively high molecular weight polymers from oxidation reaction processes. The oxidation of biodiesel also produces organic acids that would increase the acid number (Lebedevas et al., 2013). Consequently, the increased viscosity would negatively affect the fluidity and injection spray characteristics of the fuel, and the high acid number would lead to severe corrosion of fuel supply system in diesel engines (Atabani et al., 2012). Typically, oxidative stability is characterized by induction time (in hours) or oxidative stability index (OSI). Induction time is the time elapsed between starting to pass air through samples and the appearance of secondary oxidation products (volatile organic acids). A long induction time indicates favourable oxidative stability. In this study, the oxidative stability of camelina biodiesel with a value of 1.9h did not meet the standards of 3h and 8h specified in the ASTM D6751 or EN 14214:2014 respectively. Poor oxidative stability of camelina biodiesel was also observed in other studies, for example, 1.3h (Ciubota-Rosie et al., 2013), 0.6h (Soriano and Narani, 2012) and 2.5h (Moser and Vaughn, 2010). Obviously, the high percentage of polyunsaturated fatty acid esters in camelina biodiesel is the major contributor to its poor oxidative stability (Zuleta

et al., 2012; Knothe, 2007). This observation is not surprising as most biodiesels have a relatively low oxidative stability due to the existence of significant amounts of double bonds compared to petrodiesel. The common practice, the addition of small quantities of antioxidants or blending with petrodiesel can effectively solve this problem (Dunn, 2005; Liang et al., 2006). Furthermore, with the rapid advancement of research on genetic engineering and/or breeding, the fatty acid composition of camelina could be engineered to contain less unsaturated fatty acids (Kang et al., 2011).

3.5 Conclusions

The research presented in this chapter has demonstrated that it was feasible to produce biodiesel from *Camelina sativa* grown in Nova Scotia. The oil yield is in the range of 450–600 kg/ha. Camelina oil can be readily converted into biodiesel using an alkali-catalyzed transesterification process. Under the optimized reaction conditions, the maximum conversion rate reached 96%. The fuel properties were thoroughly characterized and most of these parameters complied with the standards specified in the ASTM D6751 and EN14214. However, the oxidative stability of camelina biodiesel was poor, with an induction time of 1.9h compared to the limit of 3h specified in the ASTM D6751 standards. This is attributed to a high percentage of polyunsaturated fatty acid esters in camelina biodiesel, especially linoleic acid (19.1 wt.%) and linolenic acid (33.5 wt.%). Further research is necessary to improve the oxidative stability through adding antioxidants and identifying the suitable dosages.

3.6 Acknowledgment

The authors acknowledge the financial support from the Department of Agriculture, Nova Scotia, through Growing Forward 2 Research Acceleration Program.

3.7 Transition Section

Chapter 3 has illustrated a high feasibility of biodiesel production from *Camelina sativa* oil. Most of the fuel properties of camelina biodiesel were in agreement with standard specifications and a conversion rate of 96 wt. % was obtained. In order to further evaluate the effects of independent factors and their interaction effects on camelina biodiesel

synthesis process and to maximize the camelina biodiesel yield, a statistical tool for the optimization of physical and chemical processes, response surface methodology (RSM) was applied and research results were presented in Chapter 4.

CHAPTER 4: THE OPTIMIZATION OF ALKALI-CATALYZED BIODIESEL PRODUCTION FROM *CAMELINA SATIVA* OIL USING A RESPONSE SURFACE METHODOLOGY

Contribution statement:

I was responsible for raw materials collection, experiment design and conduction, sample and data analysis and manuscript preparation.

4.1 Abstract

Camelina sativa oil is considered as a promising feedstock for biodiesel production. Response surface methodology (RSM) was used to optimize camelina biodiesel production by an alkali-catalyzed transesterification process. The effects of independent factors (temperature, time, molar ratio of methanol/oil, and catalyst concentration) on dependent variables (product yield and FAME yield), was investigated. Mathematical regression models were developed for prediction of the biodiesel product yield and FAME yield. The camelina biodiesel product yield (97%) and FAME yield (98.9%) were achieved at the optimal reaction conditions of 38.7°C reaction temperature, 40 min reaction time, 7.7 molar ratio of methanol/oil, and 1.5 wt.% catalyst concentration.

4.2 Introduction

Biodiesel, a renewable, biodegradable and environmentally innocuous biofuel, has shown great potential to be used as a substitute for conventional petrodiesel. It is typically defined as a mixture of fatty acid alkyl esters obtained through a transesterification process, in which triglycerides from vegetable oils, animal fats, and even waste cooking oils react with alcohol in the presence of a catalyst. The cost of feedstock oil accounts for 75-85% of total biodiesel production costs; thus, it is important to develop non-edible and/or low-cost

oil crops that meet certain requirements, including low agricultural inputs, high oil yield, and favorable fatty-acid compositions (Abdulla and Ravindra, 2013; Moser and Vaughn, 2010), to increase the overall economic viability of biodiesel production.

Camelina (*Camelina sativa* L. Crantz,), known as false flax or gold-of-pleasure, has recently been recognized as a promising and sustainable oilseed crop for biodiesel production in North America (Krohn and Fripp, 2012; Urbaniak et al., 2008) due to its fairly high oil content (35-43% on a dry matter basis) (Gugel and Falk, 2006; Jiang et al., 2014) and desirable plant physiological characteristics (short growing season and tolerance to drought, cool weather and insect pests) (Iskandarov et al., 2014; Kirkhus et al., 2013).

However, there is limited research on the synthesis of camelina biodiesel and the optimization of its production process (Patil et al., 2009; Wu and Leung, 2011). Many relevant studies have focused on feedstocks such as soybean, canola, and sunflower, etc. (Leung et al., 2010; Nigam and Singh, 2011; Rashid and Anwar, 2008). In particular, most of the studies on optimizing biodiesel production used a stepwise method (changing one separate factor at one time), which is not capable of assessing the interaction between each factor in the transesterification process (Leung and Guo, 2006; Mootabadi et al., 2010).

In this chapter, camelina oil was converted into biodiesel through an alkali-catalyzed transesterification process, the effects of various reaction parameters on the yield/quality of the resulting biodiesel were investigated, and the optimal reaction conditions were obtained within our experimental scope. Response surface methodology (RSM), a powerful tool in the optimization of physical and chemical processes (Awad et al., 2013; Wan Omar and Saidina Amin, 2011), was employed to evaluate the effect of independent factors on the reaction response and to determine the maximum reaction response under the optimal reaction conditions. To our knowledge, no such investigation has been reported yet. The outcomes from the present study would offer helpful knowledge in the future scale-up process for camelina biodiesel production.

4.3. Materials and Methods

4.3.1 Materials

Camelina oil used for biodiesel synthesis was cold pressed from *Camelina sativa* L. Crantz CDI007 seeds grown in Canning, Nova Scotia, Canada. Potassium hydroxide

(>85%) in the form of pellet, analytical grade methanol (>99%), calcium chloride anhydrous and hexane (>99%) were purchased from Fisher Scientific Ltd., Canada A standard reference solution of camelina methyl esters (GLC 937, >99%) was purchased from Nu-Chek Prep. Inc. USA.

4.3.2. Transesterification Process

Please refer to the Section 3.3.3.

4.3.3 Product Analysis

There are two ways to express the yield of biodiesel obtained from a transesterification process: product yield and FAME yield. The product yield shown in Eq. (1) indicates the quantity of the biodiesel produced with respect to the raw oil feed. The FAME yield in Eq. (2) is determined by the amount of FAME on the resulting biodiesel, which is an indicator of the quality of the biodiesel.

$$\text{Product yield (\%)} = \frac{\text{mass of biodiesel}}{\text{mass of oil}} \times 100\% \quad (1)$$

$$\text{FAME yield (\%)} = \frac{\text{mass of FAME}}{\text{mass of biodiesel}} \times 100\% \quad (2)$$

The FAME yield was determined by using an Agilent 7890A gas chromatography (GC) equipped with a flame ionization detector (FID) and an Agilent DB-23 column (50%-Cyanopropyl-methylpolysiloxane; 30-m length × 0.25-mm internal diameter × 0.25-µm thickness; high polarity). The carrier gas was helium and the oven temperature was initially set at 190°C and then was increased to 250°C at a heating rate of 40°C/min, remaining at 250°C for 3.5 min.

Sample preparation for GC was as follows: 25 mg of biodiesel was dissolved in 1 mL of hexane solvent, and 1 uL of the sample solution was injected into the GC with a split ratio of 40:1 for FAME identification and quantification. The fatty acid methyl esters were identified by comparing their specific retention times to those of a standard reference solution of camelina methyl esters.

4.3.4 Experimental Design

Central composite design (CCD), one of the most commonly used response surface methodology designs, was applied in this optimization study. The four independent variables were reaction temperature (°C), reaction time (min), molar ratio of methanol/oil, and catalyst concentration (wt.% with respect to oil). Three levels for each variable were determined based on our preliminary experiments as well as relevant research reported in the literature. The coded symbols, ranges, and levels of the four independent variables are given in Table 4.1. The product yield and FAME yield were selected as responses for assessing the effect of each variable, interactions between variables and optimizing experimental conditions. This three-level-four-factor CCD design generated 31 experiment combinations, including 7 center points, 8 axial points and 16 fact points. All of these combinations were replicated twice. The experimental data thus obtained were analyzed via Design-Expert version 6.0.2 and then fitted to the following second-order polynomial equation in Eq. (3) (Box and Draper, 1987; Myers et al., 2009):

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i \chi_i + \sum_{i=1}^{k} \beta_{ii} \chi_i^2 + \sum_{i} \sum_{<j=2} \beta_{ij} \chi_i \chi_j \quad (3)$$

where y is the response, χ_i and χ_j are the coded independent variables, β_0 is the constant intercept coefficient, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient. A 95% significance level was used for the analysis of variance (ANOVA) to select the model terms. The three-dimensional response surface plots were obtained as well.

Table 4.1 Independent factors and levels used for the face-centered composite design.

Variables	Symbol code	Levels		
		-1	0	1
Temperature (°C)	X1	30	40	50
Time(min)	X2	20	30	40
Methanol/oil molar ratio	X3	6:1	8:1	10:1
Catalyst concentration (wt.%)	X4	0.75	1.25	1.75

4.4. Results and Discussion

4.4.1 Regression Model Development and ANOVA Analysis

In a transesterification process, the quantity and quality of the resulting biodiesel are influenced by a number of variables, mainly including the reaction temperature, reaction time, molar ratio of methanol/oil, and catalyst concentration. Table 4.2 lists the complete design matrix (31 experimental runs) and the corresponding response data (product yield and FAME yield) based on these four independent variables (temperature, time, molar ratio of methanol/oil, and catalyst concentration). The experimental values were data obtained from experiments, and the predicted values were generated from the mathematical regression models. The quadratic regression model was suggested by Design-Expert for the two responses, and the following regression models were developed:

The product yield regression model for the coded levels is expressed in Eq. (3):

$$Y_{\text{Product yield}} = 96.02 + 1.12X_1 + 1.15X_2 + 0.44X_3 + 1.39X_4 - 0.86X_1^2 - 0.08X_2^2 - 0.13X_3^2 - 1.19X_4^2 - 1.08X_1X_2 - 0.68X_1X_3 - 2.57X_1X_4 - 0.88X_2X_3 - 1.14X_2X_4 - 0.64X_3X_4$$

The FAME yield regression model for the coded levels is described in Eq. (4):

$$Y_{\text{FAME yield}} = 97.33 + 1.70X_1 + 0.76X_2 + 1.11X_3 + 3.54X_4 - 1.05X_1^2 + 0.12X_2^2 - 0.63X_3^2 - 2.23X_4^2 - 0.10X_1X_2 + 0.37X_1X_3 - 1.95X_1X_4 - 0.26X_2X_3 - 0.61X_2X_4 - 0.85X_3X_4$$

where reaction temperature = X_1, reaction time = X_2, molar ratio of methanol to oil = X_3, and catalyst concentration = X_4.

The coefficients of the regression model terms were determined by the least squares method. The significance of the linear, quadratic, and interaction model terms and their estimated coefficients are listed in Table 4.3.

To examine how well the regression models fitted the experimental data, the Fisher F-test values (F-value), p-value, lack of fit, regression coefficient R-square (R^2), and adjusted R-square (*Adj* R^2) were evaluated by the analysis of variance (ANOVA) and are summarized in Table 4.4. Generally, a well-fitted regression model indicates a successful correlation between the response and independent variables (Jaliliannosrati et al., 2013). As seen in Table 4.4, F values of 19.89 and 21.93 for the two models, were both greater than 3.19, and the p-values were lower than 0.0001, demonstrating the validity of the developed quadratic models.

Table 4.2 The central composite design (face-centered) of four independent factors and the corresponding experimental and predicted values of responses.

No.	X1	X2	X3	X4	Temp (°C)	Time (min)	Molar ratio	Catalyst (wt.%)	Product yield (%)		FAME yield (%)	
									Exp. value	Predicted value	Exp. value	Predicted value
1	0	0	0	-1	40	30	8	0.75	92.5	92.72	92.8	91.55
2	+1	-1	-1	-1	50	20	6	0.75	92.7	92.85	88.8	89.80
3	-1	-1	+1	-1	30	20	10	0.75	86.7	87.24	85.0	86.73
4	0	0	0	+1	40	30	8	1.75	95.5	95.50	97.7	98.63
5	0	0	0	0	40	30	8	1.25	95.3	96.02	97.8	97.33
6	0	+1	0	0	40	40	8	1.25	96.1	97.08	98.3	98.21
7	+1	+1	+1	+1	50	40	10	1.75	91.2	90.15	97.7	97.25
8	-1	-1	-1	-1	30	20	6	0.75	81.0	81.96	83.7	83.02
9	-1	+1	-1	+1	30	40	6	1.75	96.1	97.37	97.8	97.95
10	+1	-1	-1	+1	50	20	6	1.75	94.1	94.03	97.1	95.89
11	+1	+1	+1	-1	50	40	10	0.75	95.3	96.06	96.8	96.99
12	+1	-1	+1	+1	50	20	10	1.75	93.2	94.06	96.7	97.67
13	+1	0	0	0	50	30	8	1.25	94.7	96.27	96.8	97.98
14	-1	-1	-1	+1	30	20	6	1.75	94.2	93.42	95.9	96.92
15	+1	+1	-1	-1	50	40	6	0.75	97.4	97.01	92.8	92.86
16	+1	-1	+1	-1	50	20	10	0.75	96.8	95.42	96.3	94.97
17	0	0	0	0	40	30	8	1.25	95.5	96.02	98.1	97.33
18	-1	-1	+1	+1	30	20	10	1.75	95.8	96.16	98.5	97.24
19	0	0	0	0	40	30	8	1.25	96.3	96.02	98.9	97.33
20	+1	+1	-1	+1	50	40	6	1.75	94.2	93.66	97.0	96.51
21	-1	+1	+1	+1	30	40	10	1.75	96.7	96.57	97.0	97.22

No.	X1	X2	X3	X4	Temp (°C)	Time (min)	Molar ratio	Catalyst (wt.%)	Product yield (%)		FAME yield (%)	
									Exp. value	Predicted value	Exp. value	Predicted value
22	0	0	0	0	40	30	8	1.25	96.7	96.02	96.6	97.33
23	0	0	-1	0	40	30	6	1.25	95.2	95.44	95.8	95.59
24	-1	+1	+1	-1	30	40	10	0.75	92.2	92.20	89.1	89.15
25	0	0	0	0	40	30	8	1.25	96.3	96.02	95.7	97.33
26	0	-1	0	0	40	20	8	1.25	95.6	94.79	97.0	96.69
27	0	0	+1	0	40	30	10	1.25	96.4	96.33	97.9	97.81
28	0	0	0	0	40	30	8	1.25	96.9	96.02	96.7	97.33
29	-1	0	0	0	30	30	8	1.25	95.5	94.04	96.1	94.58
30	-1	+1	-1	-1	30	40	6	0.75	91.3	90.45	86.2	86.49
31	0	0	0	0	40	30	8	1.25	95.6	96.02	96.5	97.33

Temp: temperature; Molar ratio: molar ratio of methanol to oil; Catalyst: catalyst concentration; min: minute

Table 4.3 The analysis of variance (ANOVA) and estimated regression coefficients for the product yield and the FAME yield models.

Factor	Product yield (%)				FAME yield (%)			
	Coefficient estimated	Standard error	F-value	P-value	Coefficient estimated	Standard error	F-value	P-value
Intercept	96.02	0.31			97.33	0.37		
Linear								
X_1	1.12	0.25	19.95	0.0004	1.70	0.30	33.02	< 0.0001
X_2	1.15	0.25	20.99	0.0003	0.76	0.30	6.62	0.0204
X_3	0.44	0.25	3.16	0.0944	1.11	0.30	14.09	0.0017
X_4	1.39	0.25	30.87	< 0.0001	3.54	0.30	142.94	< 0.0001

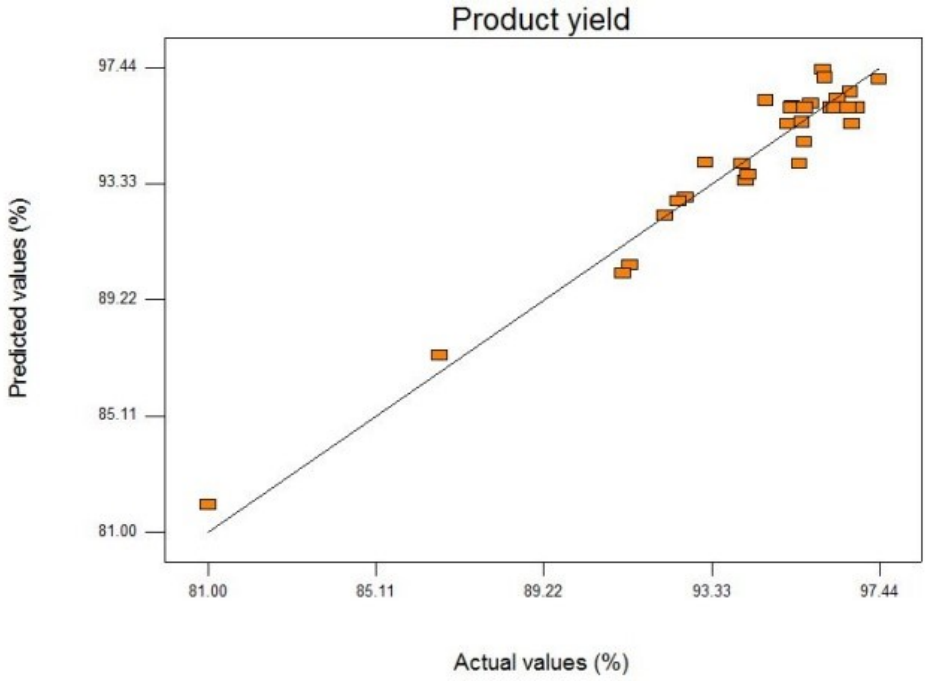
Factor	Product yield (%)				FAME yield (%)			
	Coefficient estimated	Standard error	F-value	P-value	Coefficient estimated	Standard error	F-value	P-value
Quadratic								
X_1^2	-0.86	0.66	1.70	0.2103	-1.05	0.78	1.80	0.1981
X_2^2	-0.08	0.66	0.01	0.9054	0.12	0.78	0.02	0.8769
X_3^2	-0.13	0.66	0.04	0.8466	-0.63	0.78	0.65	0.4336
X_4^2	-1.91	0.66	8.38	0.0105	-2.23	0.78	8.19	0.0113
Interaction								
X_1X_2	-1.08	0.27	16.63	0.0009	-0.10	0.31	0.11	0.7501
X_1X_3	-0.68	0.27	6.49	0.0215	0.37	0.31	1.37	0.2595
X_1X_4	-2.57	0.27	93.76	< 0.0001	-1.95	0.31	38.66	< 0.0001
X_2X_3	-0.88	0.27	11.09	0.0042	-0.26	0.31	0.69	0.4191
X_2X_4	-1.14	0.27	18.34	0.0006	-0.61	0.31	3.77	0.0701
X_3X_4	-0.64	0.27	5.76	0.0289	-0.85	0.31	7.28	0.0158

Table 4.4 The fit summary of the product yield and the FAME yield regression models.

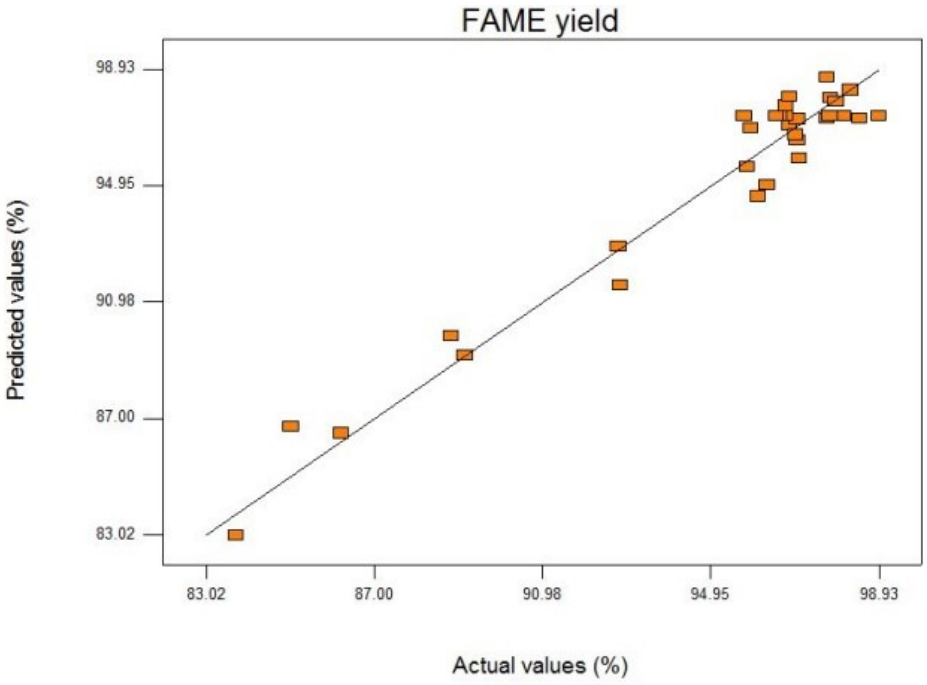
	F value	Prob>F	Lack of fit	R-Square	*Adj* R-Square
Product yield	19.89	<0.0001	0.0521	0.9457	0.8981
FAME yield	21.93	<0.0001	0.3321	0.9505	0.9071

Alternatively, the lack of fit that compares the residual error to the pure error is another good indication of the model validity. Generally, a regression model exhibits a lack of fit when it cannot adequately describe the relationship between the dependent variable and the independent factors. Therefore, a non-significant lack of fit is desirable. Here, the lack of fit for product yield and FAME yield was 0.0520 >0.05 and 0.3321>0.05, respectively, indicating that the lack of fit of the two regression models was not significant and the models fitted well to the experimental data. As for the regression coefficient R^2, the closer to 1 it is, the better the model fits the experimental data. *Adj* R^2 is the adjusted version of R^2 and it is commonly used to represent the strength of correlation between the predicted values determined by the regression models and the actual values from the experimental runs (Ceylan et al., 2008). In our work, both the *Adj* R^2 values for the product yield (0.8981) and the FAME yield (0.9071) implied a strong correlation between the predicted data and the experimental data. Fig. 4.1 (a) and (b) further depicted how well the observed values of the two responses fitted to their corresponding predicted values against the regression line (with the slope of 1).

Based on the above-combined facts, it is concluded that the quadratic regression models developed for both product yield and FAME yield were valid and showed a satisfactory correlation between the responses and the independent variables.



(a)



(b)

Fig. 4.1 (a) Actual values vs. predicted values for product yield. (b) Actual values vs. predicted values for FAME yield.

4.4.2 Effect of Process Parameters and Optimization

4.4.2.1 Effect of Linear and Quadratic Model Terms

It has been proven that a positive model term coefficient reveals the synergistic effect while a negative term implies the antagonistic effect in a transesterification process (Jaliliannosrati et al., 2013). As the linear regression coefficients of the reaction temperature, reaction time, molar ratio of methanol/oil and catalyst concentration as presented in Table 4.3, were positive, all of these four independent factors exhibited the enhancement on the product yield and FAME yield. As for the p-value of each model term, the lower it is, the more significant the model term is to its regression model (Fan et al., 2010; Wu and Leung, 2011). For this study, in the product yield regression model, only the molar ratio of methanol/oil was a non-significant model term as its p-value of 0.0944 was greater than 0.05. The order of the significance of its linear-model terms was: catalyst concentration> time > temperature > molar ratio of methanol to oil. For the FAME yield, the p-values of all four factors were less than 0.05, implying their significant contributions to the yield increase. The order of the significance of the linear terms in the FAME yield regression model was: catalyst concentration > temperature > molar ratio of methanol to oil > time. From these observations, it is evident that catalyst concentration was the primary determining factor impacting both the camelina biodiesel product yield and the FAME yield. This is in agreement with research conducted by Wu and Leung, (2011), in which an orthogonal experimental design was used to optimize biodiesel production from camelina oil. Bautista et al., (2009) also reported that KOH catalyst concentration was the most important factor for both the product and FAME yields of biodiesel derived from used cooking oil. As for the quadratic model terms, both the product yield and the FAME yield were significantly influenced only by the square of the catalyst concentration (X_4^2) and much less by other factors such as temperature, time, and molar ratios of methanol/oil.

4.4.2.2 The Interaction Effect on the Product Yield

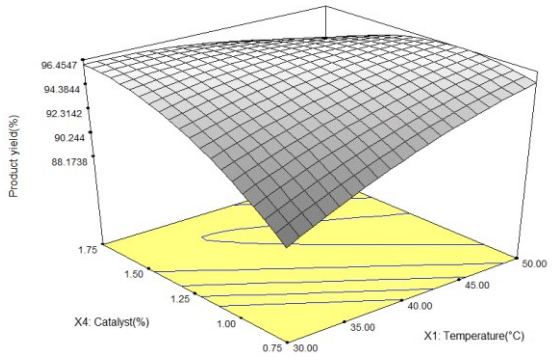
As seen from Table 4.3, the p-values of all of the interaction terms in the product yield regression model were less than 0.05, indicating that the interaction between the independent factors significantly influenced the camelina biodiesel product yield within

the experimental range. Their negative coefficients resulted in the negative contributions to the product yield. The interaction between the catalyst concentration and the temperature was the most significant one among these six interaction terms, with the lowest p-value <0.0001 and the highest estimated coefficient of -2.57. Fig. 4.2 plotted the response, product yield, as a function of two factors at one time while keeping the other two factors at a constant central point level in a three-dimensional response surface with the contour plot at the bottom.

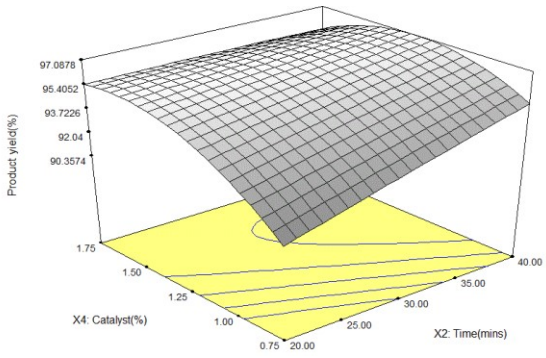
Fig. 4.2-A plots the product yield as a function of the catalyst concentration and temperature, and shows a strong interaction between these two factors. It clearly indicates that increasing catalyst concentration at a relatively low temperature range (<40°C) led to a significant increase in biodiesel product yield from approximately 88% to 96%. However, when the reaction temperature was at a relatively high level, the addition of catalyst over 1.25 wt. % resulted in a decline in the product yield. Similar patterns were observed when increasing the temperature at relatively low or high catalyst concentrations. Therefore, a significant interaction between catalyst concentration and temperature existed.

Fig. 4.2-B presents the interaction effect between the reaction time and the catalyst concentration. Increasing the reaction time from 20 min to 40 min induced the product yield increment from 90% to 97% when the catalyst concentration was at low levels. When the catalyst concentration was increased to the range of 1.3 wt. % to 1.75 wt. %, the product yield did not remarkably increase with the increased time. Therefore, the catalyst concentration and the time interacted with each other during the transesterification process and generated a significantly negative impact on the camelina biodiesel product yield (p-value of 0.0006; estimated coefficient of -1.14). This observation is consistent with other studies (Fan et al., 2010), which also reported that the interaction between the catalyst (NaOH) concentration and the reaction time significantly decreased the product yield of biodiesel derived from cottonseed oil. Comparable interaction patterns were observed as well from Fig. 4.2-C, D, E and F. In this study, therefore, it is safe to draw the conclusion that the product yield of camelina biodiesel was significantly impacted and decreased by the interactions between experimental factors.

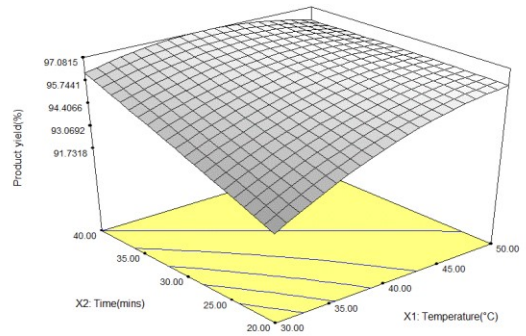
(A) Time=30 min; Molar ratio=8:1



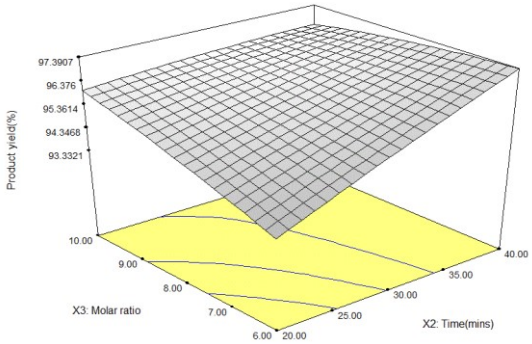
(B) Temperature=40°C; Molar ratio=8:1



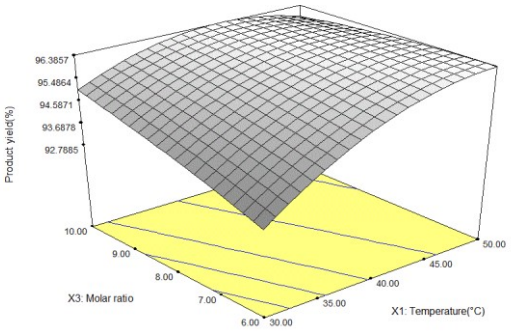
(C) Catalyst=1.25 wt.%; Molar ratio=8:1



(D) Catalyst=1.25 wt.%; Temperature=40°C



(E) Catalyst=1.25 wt.%; Time=30 min



(F) Temperature=40°C; Time=30 min

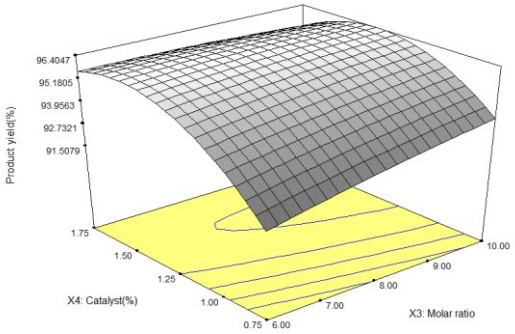


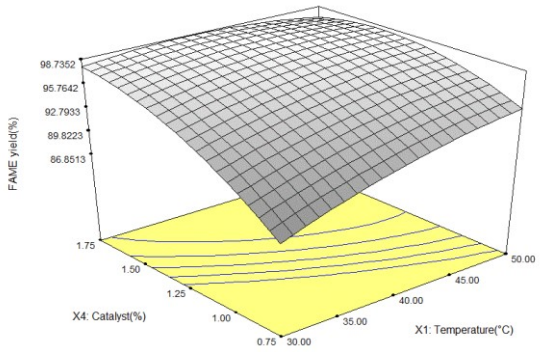
Fig. 4.2 The response surface plot of the camelina biodiesel product yield at different levels of experimental factors. (A) catalyst concentration and temperature; (B) catalyst concentration and time; (C) temperature and time; (D) time and molar ratio; (E) temperature and molar ratio; (F) catalyst and molar ratio.

4.4.2.3 The Interaction Effect on the FAME Yield

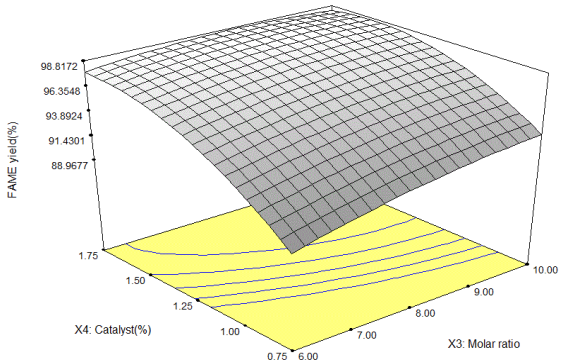
Unlike the product yield, which was significantly affected by all of the six possible interactions between the independent factors, FAME yield was remarkably influenced only by two interactions, namely the interaction between the catalyst concentration and the temperature (p-value<0.0001) and the interaction between the catalyst concentration and the molar ratio of methanol/oil (p-value of 0.0158<0.05). The interaction between the catalyst concentration and the temperature impacted the FAME yield negatively as shown in Fig. 4.3-A. Fig. 4.3-B illustrated the effect resulting from the interaction between the catalyst and the molar ratio. The FAME yield was continuously increased from 88% to 95% with the increase of the molar ratio in the range of low catalyst concentration. When the catalyst concentration was relatively high (>1.25 wt.%), increasing molar ratio of methanol/oil from 6:1 to 8:1 led to an increase in the FAME yield, but a further increase of the molar ratio slightly reduced the FAME yield.

Compared to Fig. 4.3-A and B, Fig. 4.3-C, D, E and F showed very different interaction patterns. For instance, Fig. 4.3-C exhibited a non-significant interaction between the time and temperature. The FAME yield was continuously raised by increasing time at any point of temperature within the experimental scope. The increase in the temperature also led to the FAME yield improvement when the reaction time was in the range of 20 min to 40 min. Therefore, there was a weak interaction between the time and temperature, and a negligibly adverse effect of it on the FAME yield.

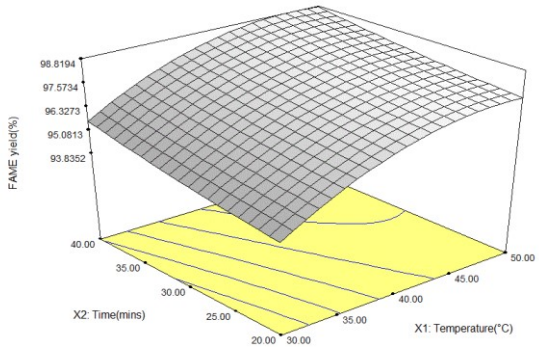
(A)Time=30 min; Molar ratio=8:1



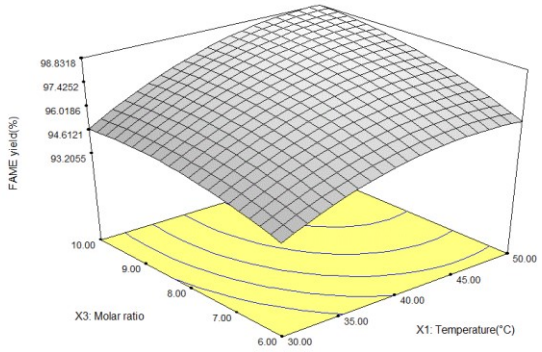
(B) Temperature=40°C; Time=30 min



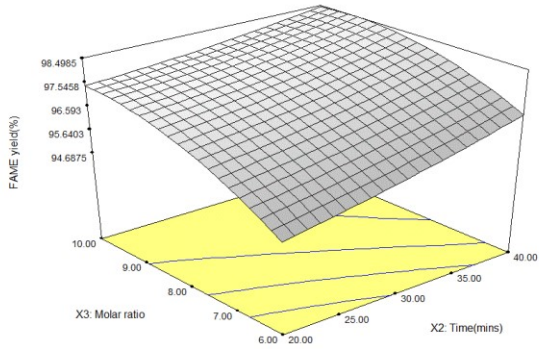
(C) Catalyst=1.25 wt.%; Molar ratio=8:1



(D) Catalyst=1.25 wt.%; Time=30 min



(E) Temperature=40°C; Catalyst=1.25 wt.%



(F) Temperature=40°C; Molar ratio=8:1

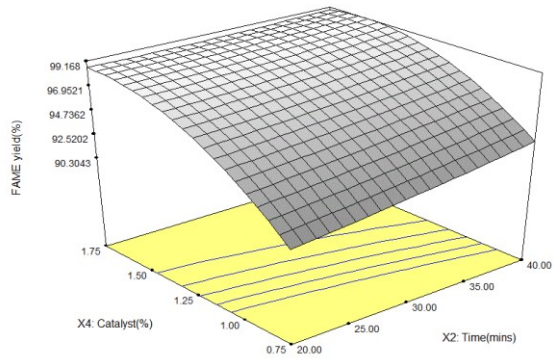


Fig. 4.3 The response surface plot of the camelina biodiesel FAME yield at different levels of experimental factors. (A) catalyst concentration and temperature; (B) catalyst concentration and molar ratio; (C) temperature and time; (D) temperature and molar ratio; (E) time and molar ratio; (F) catalyst concentration and time.

4.4.3 Optimization of Biodiesel Yields Based on the Developed Models

Aiming to achieve the maximum camelina biodiesel product and FAME yields, the optimal reaction conditions were determined by Design-Expert as follows: reaction temperature of 38.7 °C, reaction time of 40 min, molar ratio of methanol/oil of 7.7 and catalyst concentration of 1.5 wt. % with respect to raw oil. The predicted camelina biodiesel product yield and FAME yield were 97% and 98.9%, respectively. Experiments were conducted in duplicate under such optimized conditions. A good agreement between the experimental and model-determined values was achieved, which further confirmed the accuracy of the developed regression models.

To the best of our knowledge, this study is the first one that optimized alkali-catalyzed biodiesel synthesis using response surface methodology. It is worth to compare the result to results reported in literature using other feedstock such as canola, sunflower and used cooking oil (Leung and Guo, 2006; Patil and Deng, 2009; Rashid et al., 2008; Rashid and Anwar, 2008) as well as alternative optimization methodology (Wu and Leung, 2011). As shown in Table 4.5, the optimal reaction condition obtained from the present study resulted in relatively high product and FAME yield with the lowest applied temperature and largest catalyst consumption.

Table 4.5 Comparison between various studies on alkali-catalyzed transesterification of different feedstock.

Feedstock	Temp (°C)	Time (min)	Molar ratio	Catalyst (wt.%)	Product yield(%)	FAME yield(%)	Reference
Rapeseed	65	120	6:1	KOH: 1.0	95-96	-	(Rashid and Anwar, 2008)
Sunflower	60	120	6:1	NaOH: 1.0	97.1	-	(Rashid et al., 2008)
Canola	60	60	9:1	KOH: 1.0	80-95	-	(Patil and Deng, 2009)
UFO	60	20	7:1	NaOH: 1.1	88.8	-	(Leung and Guo, 2006)
Camelina	50	70	8:1	KOH: 1.0	95.8	98.4	(Wu and Leung, 2011)
Camelina	38.7	40	7.7:1	KOH: 1.5	97	98.9	Current study

UFO: used cooking oil; Temp: temperature; Molar ratio: molar ratio of methanol to oil; Catalyst: catalyst concentration. KOH: potassium hydroxide; NaOH: sodium hydroxide.

4.5 Conclusions

RSM was an effective tool to optimize the alkali-catalyzed transesterification of camelina oil under different reaction conditions (temperature, time, molar ratio of methanol/oil and catalyst concentration). Mathematical regression models of camelina biodiesel product yield and FAME yield were developed. ANOVA analysis verified the validity of the developed regression models, and also demonstrated that the catalyst concentration was the most significant factor for both product yield and FAME yield. The optimal conditions were determined to be a reaction temperature of 38.7 °C, 40 min of reaction time, 7.7 of molar ratio of methanol/oil, and 1.50 wt.% of catalyst concentration. At such optimal conditions, the maximum camelina biodiesel product yield of 97% and FAME yield of 98.9% were achieved.

4.6 Acknowledgments

The authors would like to acknowledge the financial support from the Department of Agriculture, Nova Scotia, through the Growing Forward 2 Research Acceleration Program. The authors are grateful to Jili Li for the GC operation, Dr. Ashutosh Singh for his assistance with statistical analysis and David Gibson for the biodiesel synthesis.

4.7 Transition Section

Chapter 4 demonstrated that RSM was a useful tool to optimize alkali-catalyzed transesterification of *camelina sativa* oil, and high biodiesel product and FAME yields were obtained at optimal reaction conditions. After the transesterification of camelina oil, a downstream purification of crude camelina biodiesel is necessary in order to remove a number of undesirable impurities. The traditional approach to purify biodiesel at plant scale is wet washing based on the removal of water-soluble impurities. However, this wet washing purification process generates a considerable amount of wastewater and aqueous effluent that have a detrimental impact on the environment. Hence, an exploration of alternate purification method, dry washing was carried out and presented in chapter 5.

CHAPTER 5: A COMPARATIVE STUDY ON THE PERFORMANCE OF FIBER-BASED BIO-SORBENTS IN CAMELINA BIODIESEL PURIFICATION

Current state:

A version of this chapter is under preparation for a submission to Journal of Environmental Chemical Engineering.

Contribution statement:

I was responsible for biodiesel preparation, experiment design and conduction, sample and data analysis and manuscript preparation.

5.1 Abstract

Biodiesel has received great interest for use as a promising alternative for traditional petrodiesel. Biodiesel downstream purification is typically carried out by a wet washing process that generates large amounts of wastewater. Purifying crude biodiesel by a dry washing method, therefore, has become an emerging trend. Three dry wash media (commercially available BD-Zorb, sawdust and wood shavings) were chosen to investigate their purification performance on crude camelina biodiesel in this chapter. The soap removal capacity of three studied fiber-based biosorbents was 9.4 mL/g, 24.4 mL/g and 51.1 mL/g for wood shaving, sawdust and commercial BD-Zorb respectively when the soap content of the crude camelina biodiesel was 9007 ppm. The main mechanism of soap removal by sawdust and wood shavings were the physical filtration and adsorption. The acid number of camelina biodiesel purified by BD-Zorb was greater than 1 mg KOH/g), failing to meet the ASTM D6751 specification (<0.5 mg KOH/g) due to the presence of a small amount of resin in BD-Zorb.

5.2 Introduction

A typically used method for biodiesel production is transesterification, a process in which vegetable oils (triglycerides) react with alcohol (usually methanol) to generate a mixture of fatty acid mono-alkyl esters (FAME or biodiesel) and by-product (glycerol) in

the presence of alkaline catalysts (usually potassium hydroxide, KOH or sodium hydroxide, NaOH). The majority of glycerol can be removed from biodiesel by phase separation because of the density and polarity difference between them. However, even after the glycerol removal, crude biodiesel still contains a variety of impurities, such as alcohol, catalyst, water, glycerol, unreacted mono-, di- and triglycerides, free fatty acids and soap etc. (Atadashi et al., 2011; Stojković et al., 2014) The presence of those impurities may lead to severely negative effects on fuel quality and diesel engine as mentioned in Section 2.4 (Atabani et al., 2012; Wan Ghazali et al., 2015). Therefore, proper purification processes are essential to decrease or minimize those impurities in crude biodiesel in order to meet the specifications required by the standards of American Society for Testing and Materials (ASTM) D6751 or/and European Committee for Standardization (CEN EN14214).

The traditional approach to purify biodiesel at a commercial production scale is wet washing based on the removal of water-soluble contaminants. Although hot distilled or deionized water enables to effectively remove residual glycerol, soap, alcohol and catalyst from crude biodiesel, this wet washing purification process generates a great amount of wastewater and aqueous effluent that have a detrimental impact on the environment (Berrios and Skelton, 2008; Squissato et al., 2015). Therefore, numerous efforts have been contributed to purify crude biodiesel by dry wash media, such as resins (Wall et al., 2011), silicate magnesol (Faccini et al., 2011), activated carbon (Fadhil et al., 2012) and membranes (Gomes et al., 2013).

The adsorption capacity of cellulose fiber, a natural polymer derived from biomass with a production of 10^{11} tonnes per year, has also been extensively studied over the years (Grunin et al., 2012; Heinze and Liebert, 2001; Squissato et al., 2015). Recently, several cheap alternatives (fiber-based biosorbents), such as Eucalyptus pulp (Squissato et al., 2015) and Rice husk ash (Manique et al., 2012), have been investigated for biodiesel purification. BD-Zorb, a fiber-based dry wash media consisting mostly of wood chips and a small amount of resin, has been a commercially available product used for biodiesel purification. On the other hand, large amounts of sawdust and wood shaving waste are generated by wood processing plants annually and they might also react as efficient biosorbent for biodiesel purification.

In this chapter, taking camelina biodiesel as a model crude biodiesel, we explored the potential of using wood waste, sawdust and wood shavings as bio-sorbents in the dry washing purification processes, and compared the performance of the wood waste to that of commercially available adsorbent, BD-Zorb. The soap level, acid number and water content of purified biodiesel were indicators for evaluating the purification ability of dry wash media. This study also compared the soap removal efficiency of dry washing method to the wet washing method, and the effect of water temperature on soap removal ability during the wet washing process was also evaluated.

5.3 Materials and Methods

5.3.1 Materials

Unrefined *camelina sativa* oil used for biodiesel synthesis was cold pressed from seeds grown in Nova Scotia, Canada. Potassium hydroxide in the form of pellets, analytical grade methanol (>99%) and hexane (>99%) were purchased from Fisher Scientific Ltd., Canada. A standard reference solution of camelina methyl esters (GLC 937, >99%) was purchased from Nu-Chek Prep. Inc. USA. Three fiber-based adsorbents employed at this purification study as shown in Fig 5.1. BD-Zorb was purchased from Utah Biodiesel Supply Ltd., USA ,and sawdust and wood shavings were provided by the wood shop at Dalhousie Agricultural Campus, Truro, NS. Canada.

Fig. 5.1 Three adsorbents applied for biodiesel purification: BD-Zorb, sawdust and wood shavings.

5.3.2 Camelina Biodiesel Synthesis

Camelina biodiesel used in this study was prepared by an ultrasound-assisted transesterification process of the temperature of 50 ºC, the reaction time of 1 hour, in the presence of 8:1 methanol/oil molar ratio and 1.3 wt. % KOH catalyst. The reaction mixture was transferred to a separatory funnel and allowed to stand for 30 min for phase separation. The glycerol layer under the crude biodiesel was drawn off and remaining crude biodiesel was used for the purification process.

5.3.3 Purification Process

The purification of crude camelina biodiesel by dry wash media was performed in three cylindrical separator funnels (125 ml) filled with 18 g BD-Zorb, sawdust and wood shavings respectively. Cotton gauze was applied on the top of the packed absorbent to distribute the added crude biodiesel evenly and avoid the potential channeling phenomenon. Meanwhile, cotton gauze was also used at the bottom of the adsorbent bed to hold the mass. The flow rate of biodiesel was controlled by a rotary knob at one bed volume (the volume of 18 g biosorbent in funnel) per hour and every 50 ml of purified biodiesel was collected and stored in a beaker for further testing.

For a wet washing purification process, 50 mL of crude camelina biodiesel was transferred to a 250 mL separatory funnel and washed by adding 100 mL distilled water each time until the water layer was transparent. The temperature of distilled water was set at 20 ºC and 50 ºC in order to compare their purification efficiency. The purification operation of both dry and wet washing process were conducted in duplicate in this experiment.

5.3.4 Characterization of Camelina Biodiesel

The contents of fatty acid methyl esters (FAME) at camelina biodiesel were determined by using the same method described in Section 4.3.3. The soap level of biodiesel was determined by titration examination: 12 mL biodiesel and 1 mL 0.04% aqueous Bromophenol blue were added into 100 mL of iso-propanol. The solution was mixed well by magnetic stirring, followed by dropwise adding 0.01 N hydrochloric acid (HCl) until the color of the solution changed from blue or/and green to yellow. The amount of HCl used (mL) was recorded and the soap level of biodiesel was determined:

Soap content (ppm) = the amount of HCl added (mL) * 320

The water content and acid number of camelina biodiesel were tested by following standard method EN ISO 12937 and EN 14104 respectively.

5.4 Results and Discussion

5.4.1 The Properties of Crude Camelina Biodiesel

The crude biodiesel synthesized by a transesterification process contained 97.5 wt. % of FAME, the soap level was 9007 ppm, water content was 2011ppm, and acid number was 0.07 mg KOH/g as shown in Table 5.1.

Table 5.1 The properties of crude camelina biodiesel in this study.

Properties	ASTM D6751	EN 14214	Crude biodiesel
Soap content (ppm)	-	-	9007
Acid number **(**mg KOH/g**)**	0.5	0.5	0.07
Water content (ppm)	500	500	2011
FAME (wt. %)	-	96.5	97.5

The high-level soap resulted from a stoichiometric excess KOH (1.5 wt. %) added, and thus intense saponification reaction between FFA in parent camelina oil and KOH occurred during the transesterification process. The reaction mechanism of saponification was presented in Fig 5.2. The hydrogen atoms located in FFA could be replaced by potassium ions (K^+) from KOH and then form soap and water.

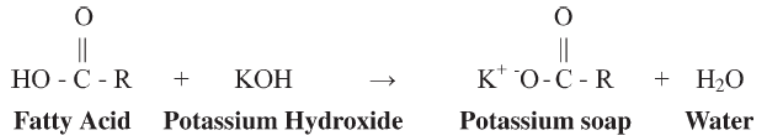


Fig. 5.2 Formation of soap between fatty acid and excessive KOH catalyst (Gerpen, 2005).

On the other hand, similar to glycerol, both soap and water remain in crude biodiesel tended to settle down slowly with time, but those properties of crude camelina biodiesel were measured right after the glycerol removal in the present study. So this could be another reason for extremely high soap and water content.

5.4.2 Purification of Crude Biodiesel Using Different Fiber-based Dry Wash Media

Crude camelina biodiesel with an initial soap level of 9007 ppm was purified by using three fiber-based adsorbents, including commercially available BD Zorb and waste products from wood processing plant (sawdust and wood shavings). Fig. 5.3 provided the comparison of soap removal efficiency of these three adsorbents. Clearly, all of them were greatly effective to remove soap and decrease the soap content of crude camelina biodiesel from the initial concentration of 9007 ppm to less than 66 ppm for the first 150 mL of purified biodiesel. However, wood shavings were not able to maintain the satisfactory soap content level (66ppm) after purifying 200 mL crude camelina biodiesel and soap content continuously increased to 99 ppm, 176 ppm and 328 ppm for 200 mL, 250 ml and 300 ml of purified biodiesel respectively. Sawdust showed a relatively better soap removal efficiency than wood shavings, as the soap level was 72 ppm after purifying 450 mL biodiesel as shown in Fig. 5.3. In comparison with wood shavings and sawdust, BD Zorb exhibited a much higher efficiency of soap removal, and the soap content was 56 ppm after treating of 900 mL crude biodiesel. A relatively slower increasing trend of the soap content in the purified biodiesel was observed for BD Zorb.

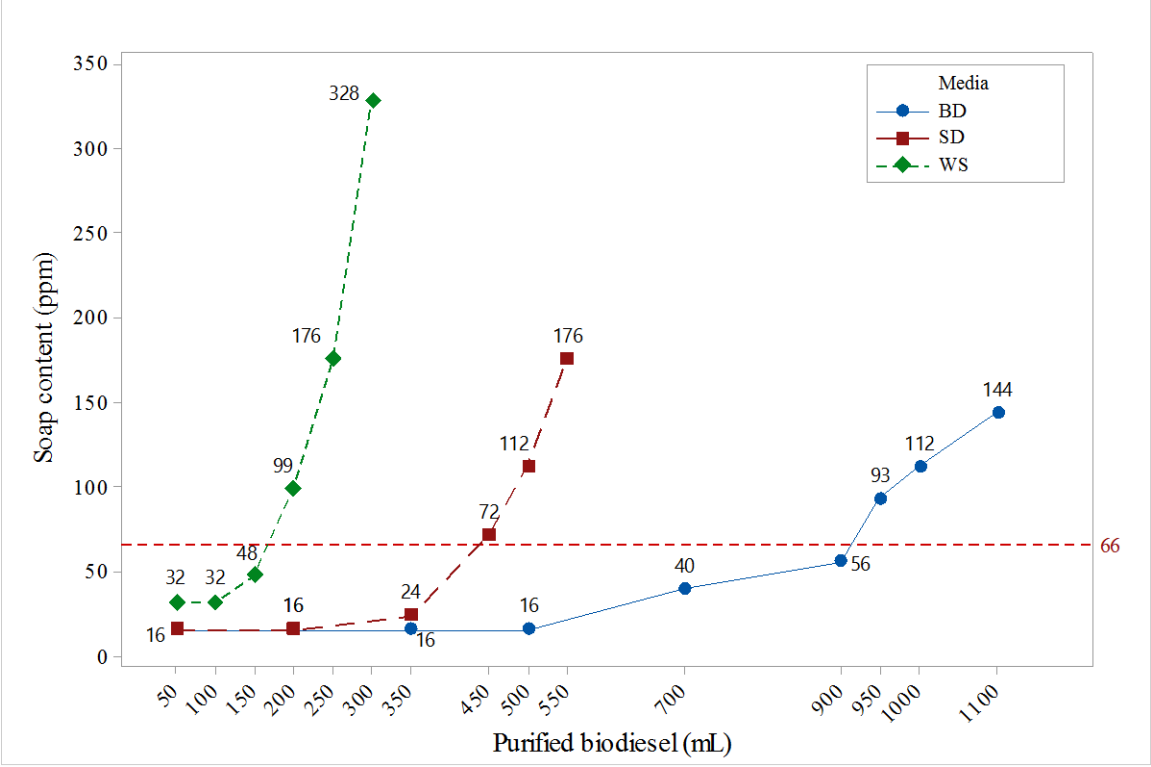


Fig. 5.3 Comparison of soap removal efficiency of each 18 g dry wash media, BD Zorb (BD), Sawdust (SD) and Wood shaving (WS).

The acid number of biodiesel is a good indicator of the free fatty acid (FFA) content in biodiesel, representing its degree of acidity. As mentioned in Section 5.4.1, excessive KOH was applied for camelina biodiesel synthesis in order to achieve high biodiesel purity in the present study. Therefore, the initial acid number of crude camelina biodiesel was extremely small (0.07 mg KOH/g). The effects of different types of dry washing media on the acid number during the purification process was shown in Fig. 5.4.

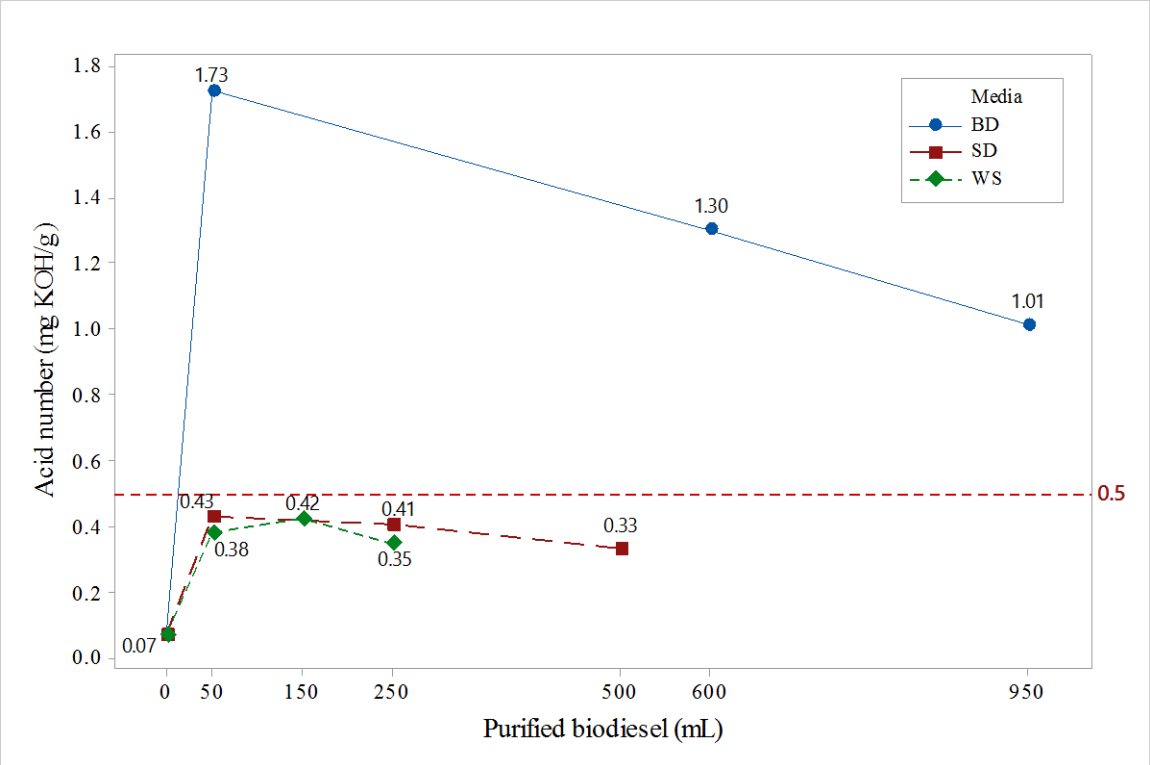


Fig. 5.4 Comparison of the acid number of purified camelina biodiesel treated by 18 g dry washing media, BD Zorb (BD), Sawdust (SD) and Wood shaving (WS).

The acid number of BD Zorb purified biodiesel at 50 mL was 1.73 mg KOH/g, which was greatly higher than the initial 0.07 mg KOH/g. This can be well explained that a small amount of resin was visually observed in BD Zorb, which removed soap from the crude biodiesel via ion exchange mechanism and generated FFA. The formed FFA led to an increased acid number of purified biodiesel when BD Zorb was used as an adsorbent. A steadily declining trend of acid number was observed as increasing the volume of the treated biodiesel, indicating the ability of BD Zorb to remove soap decreased gradually when the crude camelina biodiesel was continuously fed to the BD Zorb bed. Similar to

BD Zorb, the acid number of purified biodiesel using sawdust and wood shavings as media also increased to 0.43 mg KOH/g and 0.38 mg KOH/g respectively from the initial value of 0.07 mg KOH/g when 50 mL crude camelina biodiesel was treated, and then decreased along with further purification. Noticeably, the acid number of the biodiesel purified by sawdust and wood shavings were all less than 0.5 mg KOH/g, the limit specified in the ASTM standard, but BD Zorb purified biodiesel still had a much higher acid number (1.01 mg KOH/g) at 950 mL.

As previously noted, the resulting high acid number of the biodiesel purified by BD Zorb can be attributed to the presence of resin. The hydrogen atoms in the resin that typically contains hydroxyl groups can be exchanged with sodium or potassium ions from soap then produce FFA. This ion exchange mechanism also can be used to explain the increase in the acid number of biodiesel purified with sawdust and wood shavings. Sawdust and wood shaving are fiber-based adsorbents, which are typically composed of 40-50% cellulose, 20-30% hemicellulose and 15-30% lignin. Lignin that connects cellulose and hemicellulose are chemically cross-linked phenolic polymers. The hydroxyl groups in those phenol molecules can provide hydrogen atoms for exchanging with cations K^+ in soap, therefore generate a small amount of FFA. The acid number of biodiesel purified with sawdust and wood shaving was not as high as that of BD Zorb purified biodiesel which indicated that ion exchange was not the primary mechanism for sawdust and wood shaving to remove soap from the crude camelina biodiesel. The soap removal was primarily contributed by the physical filtration and adsorption in the purification processes using sawdust and wood shavings.

Fig. 5.5 showed the water removal efficiency of three studied dry wash media. It was found that all of them were able to filter out or/and adsorb water from the crude camelina biodiesel, and the water content was decreased from an initial water content of 2011 ppm to about 664-942 ppm when 50 mL biodiesel was purified. The water content of the purified biodiesel gradually increased with continual feeding of crude camelina biodiesel to the purification columns packed with the dry wash media. In general, BD Zorb exhibited a relatively higher water removal efficiency compared to those of wood shavings and sawdust. This could be mainly attributed to the pre-drying treatment process applied to the commercial product, BD Zorb. However, no pre-drying treatment was conducted for either

wood shavings or sawdust in the present study. It is worthy to note, the water content of the purified camelina biodiesel via dry washing were all higher than the limit 500 ppm required in the ASTM D6751 specification. Thus, these three fiber-based biosorbents were not able to remove water from the crude camelina biodiesel to a satisfactory level.

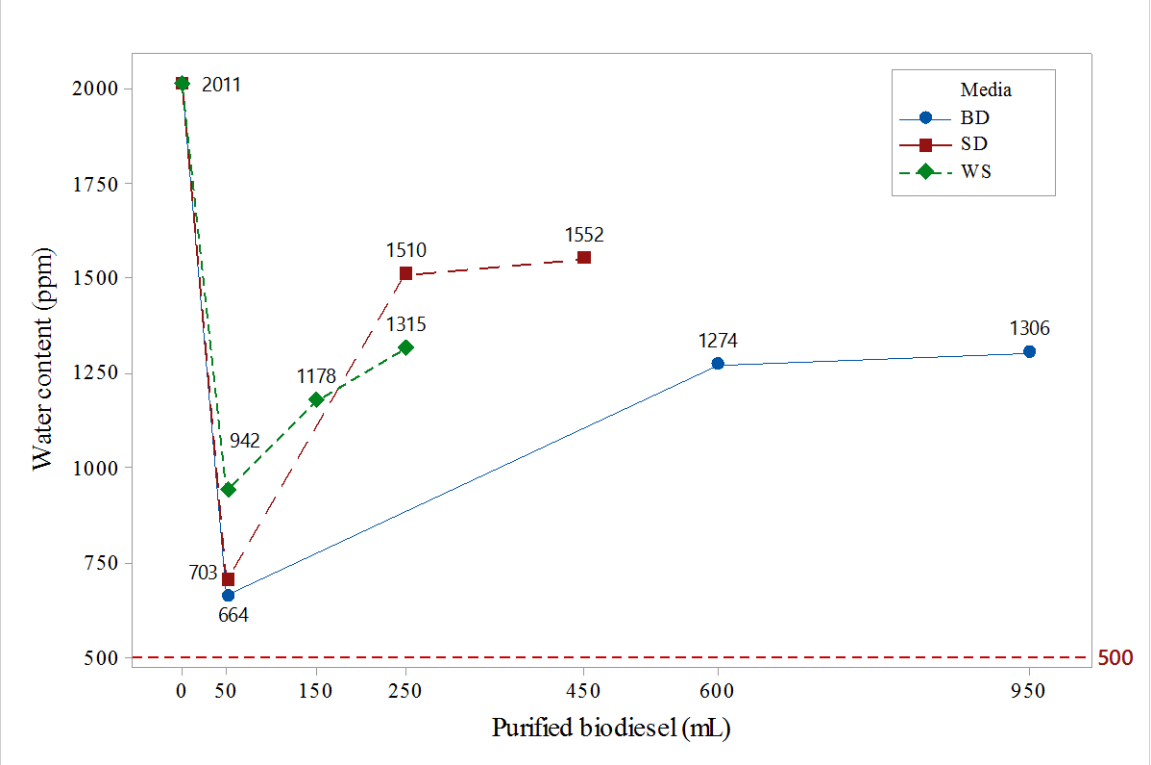


Fig. 5.5 Comparison of the water content of the purified camelina biodiesel treated by 18 g dry washing media, BD Zorb (BD), Sawdust (SD) and Wood shaving (WS).

In summary, the water content and acid number of camelina biodiesel purified by three dry washing media when the soap level was reduced from the initial concentration of 9007 ppm to 66 ppm, were presented in Table 5.2 along with the estimated soap removal capacity of three adsorbents. As shown in Table 5.2, the soap removal capacity of three studied fiber-based biosorbents was 9.4 mL/g, 24.4 mL/g and 51.1 mL/g for wood shavings, sawdust and commercially available BD-Zorb respectively.

Table 5.2 The properties of camelina biodiesel purified by dry wash media and their adsorption capacities.

Adsorbent used	Soap content (ppm)	Acid number (mg KOH/g)	Water content (ppm)	Estimated volume of biodiesel treated (mL)	Soap removal Capacity (mL biodiesel /g adsorbent)
BD	66	>1.01	Nearly 1306	920	51.1
SD	66	<0.41	Nearly 1552	440	24.4
WS	66	<0.42	Nearly 1178	170	9.4

5.4.3 Effect of Water Temperature on Soap Removal Efficiency

Wet washing was conventionally used to purify crude biodiesel in an industry-scale, and it was estimated that about 10 L wastewater could be generated by purifying 1 L crude biodiesel (Karaosmanoğlu et al., 1996; Saleh et al., 2010). Water temperature also played a crucial role on wet washing purification efficiency (Saleh et al., 2010). A comparison study of soap removal ability of 20 ºC and 50 ºC distilled water was carried out in the present study, and results are shown in Fig. 5.6.

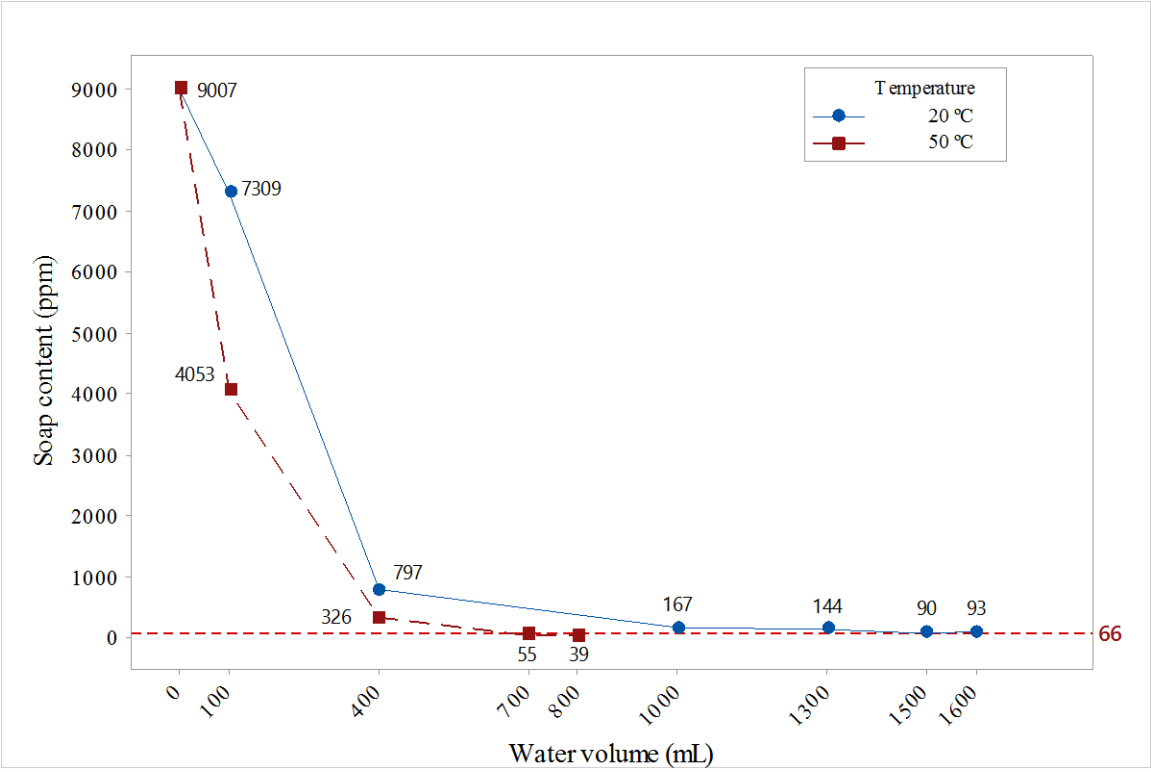


Fig. 5.6 Comparison of the soap removal efficiency between distilled water at different temperatures (20 ºC vs 50 ºC).

Taking a batch of 50 mL crude camelina biodiesel, 100 mL of distilled water at 50 ºC was added and enabled to substantially decrease the soap content from as high as 9007 ppm to 4053 ppm, however, the same amount of 20 ºC distilled water was not able to remove soap as efficiently as 50 ºC water. Soap level was gradually lowered by repeatedly washing the biodiesel using 100 mL 50 ºC water. Eventually, the soap level reached to 55 ppm (<66 ppm) until 700 mL 50 ºC distilled water was consumed, giving a ratio of 14:1 water/crude biodiesel in volume. Alternatively, using 20 ºC distilled water, the soap content of camelina biodiesel was decreased to 90 ppm until 1500 mL water used, leading to a water consumption ratio of 30:1water/crude biodiesel in volume. It is concluded that a larger amount of distilled water was needed to remove soap content to a satisfactory level when the temperature was low and increasing the water temperature resulted in better soap removal efficiency. Gonzalo et al., (2010) have also demonstrated that using higher temperature water (60 ºC) for biodiesel purification resulted in relatively better purification efficiency than lower temperature (40 ºC).

5.5 Conclusions

Purifying crude biodiesel by dry washing media is a promisingly alternative option. The work in this chapter demonstrated that three fiber-based biosorbents of interest in this chapter were able to effectively remove the soap existing in the crude camelina biodiesel to an acceptable level. The soap removal capacity was 9.4 mL/g, 24.4 mL/g and 51.1 mL/g for wood shaving, sawdust and commercially available adsorbent, BD-Zorb respectively when the initial soap content of the treated crude camelina biodiesel was 9007 ppm. Even though BD-Zorb exhibited a relatively higher soap removal capacity, the acid number of BD-Zorb purified camelina biodiesel (> 1 mg KOH/g) were much higher than the specified limit of 0.5 mg KOH/g in the ASTM D6751 standard due to the presence of a small amount of resin in BD-Zorb. The main mechanism of soap removal by sawdust and wood shavings were the physical filtration and adsorption while for the adsorbent, BD-Zorb, ion exchange mechanism also played a more important role in the soap removal. The water content of camelina biodiesel purified by the three biosorbents were all greater than the limit of 500 ppm stated in the ASTM. These results indicate that additional steps are needed to decrease the water content and acid number of biodiesel when fiber-based dry washing media are

applied. In terms of the wet washing, generally, a large amount of distilled water is needed, and the biodiesel purification efficiency is highly associated with the temperature of water used. As for 50 ºC water, the water consumption ratio was 14:1 water/biodiesel in volume, and the ratio increased to 30:1 water/biodiesel in volume if 20 ºC water was used to reduce the soap content of 9007 ppm in the crude camelina biodiesel to a satisfactory level.

5.6 Acknowledgement

The authors acknowledge the financial support from NSERC Discovery, Bo Fang for carrying out preliminary experiments, and Chris Nelson for providing wood sawdust and wood shavings.

5.7 Transition Section

As identified in chapter 3, the oxidative stability of camelina biodiesel was poor mainly because of its high percentage of unsaturated fatty acid methyl esters. Improving camelina biodiesel oxidative stability becomes extremely necessary for its commercial applications. The addition of antioxidants is a common practice to increase biodiesel resistance to autoxidation in the biodiesel industry. Therefore, chapter 6 comprehensively evaluated the effect of synthetic antioxidants on camelina biodiesel oxidation and storage stability, and aims to determine the suitable amount of the most effective antioxidants for improving camelina biodiesel anti-oxidation performance.

CHAPTER 6: THE EFFECT OF SYNTHETIC ANTIOXIDANTS ON OXIDATION AND STORAGE STABILITY OF BIODIESEL DERIVED FROM *CAMELINA SATIVA* OIL

Current state:

A version of this chapter is under preparation for a submission to the journal of Applied Energy.

Contribution statement:

I was responsible for raw material collection, biodiesel preparation, experiment design and conduction, sample and data analysis and manuscript preparation.

6.1 Abstract

Adding synthetic antioxidants is a commonly applied solution in the biodiesel industry to ensure a satisfactory biodiesel oxidative stability. Oxidative stability index (OSI) and peroxide value (PV) were used to assess the oxidative stability of antioxidant treated biodiesel derived from *Camelina sativa* oil in this chapter. The effectiveness of four commercially available antioxidants were evaluated, including butylated hydroxyanisole (BHA), 2,6-Di-*tert*-butyl-4-methylphenol (BHT), propyl gallate (PrG) and *tert*-butylhydroquinone (TBHQ). TBHQ presented the highest effectiveness at improving camelina biodiesel oxidation and storage stability. Satisfactory oxidation stability of camelina biodiesel was achieved by treating camelina biodiesel with either 2000 ppm BHT, 1000 ppm PrG or 1000 pm TBHQ. The storage stability of camelina biodiesel was evaluated under two different ageing methods as well, including a 24h accelerated ageing method and another ageing method stated in the ASTM D4625. Ageing camelina biodiesel by ASTM D4625 method seems to simulate more intensively oxidative degradation of camelina biodiesel compared to the 24h ageing method. Both the oxidation and storage stability of camelina biodiesel were improved to a satisfactory level by adding a suitable amount of TBHQ.

6.2 Introduction

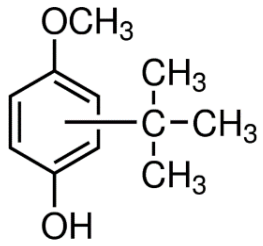
Biodiesel is generally more sensitive to oxidative degradation than petrodiesel and this has become a major drawback of biodiesel derived from vegetable oils. The reason for this is that biodiesel is inherently composed of a high percentage of unsaturated long chain fatty acid esters that is highly susceptible to oxidation (Dunn, 2008; Mittelbach and Schober, 2003). The rate of biodiesel oxidation is dependent on the number of allylic and bis-allylic sites within unsaturated fatty acid ester chains (Bouaid et al., 2007; Jain and Sharma, 2010). Oxygen is able to readily attach to those reactive allylic and bis-allylic sites, therefore, initiates the primary free-radical chain reaction and forms intermediate compounds (hydroperoxides). On the stage of the secondary oxidation, the resulting hydroperoxides are further decomposed to form volatile organic acids, alcohols, ketones, and aldehydes etc. (Zuleta et al., 2012).

The measurement of oxidative stability index (OSI) or induction period (IP) is typically used to evaluate biodiesel oxidative stability, which basically monitors the appearance of secondary products from oil degradation. OSI testing is conducted under isothermal condition (110 ºC) while steadily purging dry air stream through the oil sample, therefore, oil degradation happens and its secondary products (such as volatile organic acids) could be swept into the test tube containing deionized water and a conductivity probe. The water conductivity is periodically measured and OSI is defined as the time point where the sharp conductivity increase appears (Dunn, 2008). Alternatively, peroxide value (PV) is another commonly used parameter that assesses the amount of primary oxidation products (such as hydroperoxides) in biodiesel. Although PV provides information concerning the extent of biodiesel oxidation, it might not be suitable for monitoring biodiesel storage stability over a long period of time. This is because PV tends to increase at the early stage of oxidation and then decreases as hydroperoxides decompose to form secondary oxidation products (Dunn, 2002).

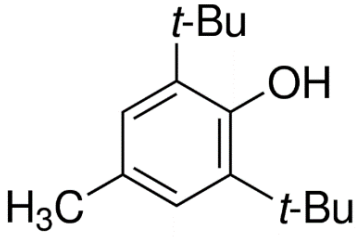
Long-term storage stability of biodiesel is defined by how well it will resist to changes caused by the external storage environment. During a long period of storage, autoxidation of biodiesel initiated by air exposure (Plessis et al., 1985), excessive metals (Knothe and Dunn, 2003) and storage tank (Bondioli et al., 1995) resulted in legitimate concerns with respect to monitoring and maintaining fuel quality. ASTM D4625 (Diesel Storage Stability

at 43 ºC) has been reported as a reliable ageing method to monitor the biodiesel quality during storage (Bondioli et al., 2002; Christensen and McCormick, 2014; Rand et al., 2010). Bondioli et al., (2002) stated that ASTM D4625 was generally recognized as the best way to simulate storage behavior. However, this ageing method is very time-consuming (4-24 weeks ageing period) which makes it is not suitable for biodiesel quality control during storage. Therefore, Bondioli et al., (2004) proposed an alternative test method to quickly predict biodiesel storage stability, and they suggested using modified Rancimat apparatus to age 3 grams of biodiesel sample for 24 hours at 80 ºC with an airflow rate of 10 L/h.

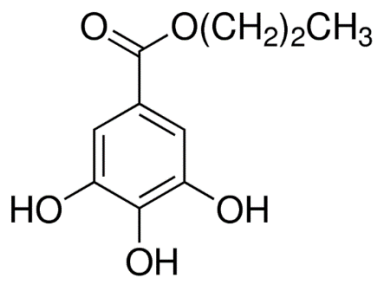
Biodiesel properties such as acid number, kinematic viscosity, OSI and PV of biodiesel are adversely or unfavorably changed by the extensive degradation during storage (Bondioli et al., 1995; Dunn, 2002); in addition, high molecular weight polymers generated from biodiesel oxidation could clog fuel lines and pumps as well (Knothe, 2007). Hence, eliminating the undesirable effect of fuel autoxidation on the biodiesel quality is critically important for biodiesel fuel producers, suppliers and consumers (Dunn, 2008). Treating biodiesel with oxidation inhibitors (antioxidants) is a commonly used and promising approach to increase the resistance against oxidation in the edible vegetable oil and biodiesel industry (Dunn, 2008; Marinova et al., 2008; Mittelbach and Schober, 2003; Rudnik et al., 2001). Synthetic antioxidants are derived from petroleum and have been utilized to improve the oxidation and storage stability of biodiesel, such as butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), *tert*-butylhydrooquinone (TBHQ) and propyl gallate (PrG) (R. Dunn, 2005; Mittelbach and Schober, 2003; Osawa et al., 2015). The chemical structures of these four antioxidants are given in Fig. 6.1.

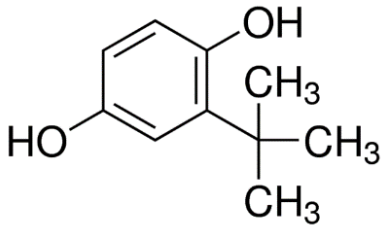


Butylated hydroxyanisole (BHA)



2,6-Di-*tert*-butyl-4-methylphenol (BHT)





Propyl gallate (PrG) *tert*-butylhydroquinone (TBHQ)

Fig. 6.1 The chemical structure of synthetic antioxidants (BHA, BHT, PrG and TBHQ).

Those synthetic antioxidants are able to terminate the free radical chain reaction and thus to delay the oxidation initiation as their active hydroxyl groups (–OH) provide protons for the oxidized free radicals and form stable radicals. Dunn, (2005) suggested that 3000 ppm BHA added to biodiesel could sufficiently minimize the auto-oxidation during a long period of storage. However, Liang et al., (2006) reported that adding only 50 ppm TBHQ was enough to obtain satisfactory oxidation stability of palm oil biodiesel. Osawa et al., (2015) demonstrated that the oxidation stability index of *Croton megalocarpus* biodiesel treated with 1000 ppm PrG and BHA only decreased by 12.2 % and 20.59% respectively after 8 weeks storage. Several reviews also illustrated that the effectiveness of different antioxidants on oxidation and storage stability of biodiesel derived from various raw materials (*e.g.* rapeseed, sunflower, soybean, palm, tallow and used cooking oil), greatly depended on the concentration of antioxidants, the degree of unsaturation of fatty acid methyl esters (FAME), storage conditions and the presence of metal etc. (Dunn, 2008; Zuleta et al., 2012; Jain and Sharma, 2010; Knothe, 2007). Supriyono et al., (2015) suggested that dedicated studies need to be conducted to determine the appropriate type and concentration of antioxidant when considering the use of antioxidants for improving oxidation and storage stability of biodiesel derived from different parent oils.

Chapter 3 has illustrated that the oxidative stability of biodiesel derived from *Camelina sativa* oil was only 1.9h at 110 ºC, which was not satisfactory in accordance with the ASTM D6751 (3 h) and EN 14214 (8 h) (Yang et al., 2016). To our knowledge, there is no research with a focus on the stability improvement of camelina biodiesel. Moreover, most studies related to the improvement of biodiesel stability only focused on either oxidation stability

or storage stability. This chapter comprehensively evaluated the effect of synthetic antioxidants (BHA, BHT, PrG and TBHQ) with varying loading (from 500 ppm to 3000 ppm) on both oxidation stability and storage stability of camelina biodiesel. Two parameters, OSI and PV were tested for assessing the stability of biodiesel, and the suitable dosage of the most effective antioxidant was determined. This research would provide valuable information for a commercial application of this emerging energy crop in the near future.

6.3 Materials and Methods

6.3.1 Materials

Camelina sativa oil was cold pressed from *Camelina sativa* L. Crantz CDI007 seeds grown in Canning, Nova Scotia, Canada. Potassium hydroxide in the form of pellet, 1 N hydrochloride (HCl) analytical grade methanol, spectroscopy grade isooctane, isopropanol and butanol were purchased from Fisher Scientific Ltd. Canada. Cumene hydroperoxides, ammonium thiocyanate, barium chloride ($BaCl_2$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), butylated hydroxyanisole (BHA), 2,6-Di-*tert*-butyl-4-methylphenol (BHT), propyl gallate (PrG), *tert*-butylhydroquinone (TBHQ) were purchased from Sigma Aldrich, Canada.

6.3.2 Synthetic Antioxidant Treatment for Camelina Biodiesel

Cold pressed camelina oil was used to produce camelina biodiesel at a laboratory scale via an alkaline-catalyzed transesterification process. A pre-calculated amount of methanol solution (8:1 molar ratio of methanol to oil) containing completely dissolved KOH (1.5 wt.% to oil) was added to camelina oil and this transesterification reaction was carried out for 1 hour at 50 ºC under ultrasound assisted condition. The reaction mixture was transferred to a separatory funnel and allowed to stand for 30 min for phase separation, and then the glycerol layer under the crude biodiesel was drawn off. The crude biodiesel remaining in the separatory funnel was washed by a few batches of warm distilled water until the water layer became completely translucent. The purity of camelina biodiesel obtained was approximately 97 % suggesting that the reaction was nearly complete.

The antioxidants (BHA, BHT, PrG and TBHQ) were dosed at concentrations of 500, 1000, 1500, 2000, 2500, 3000 ppm along with one control group (0 ppm) in synthesized camelina biodiesel. All the antioxidants were found to dissolve completely in the biodiesel at all concentrations even though the physical compatibility of PrG to biodiesel was not as good as others.

6.3.3 OSI and PV Determination

Oxidation stability of camelina biodiesel samples with varying dosage of antioxidants was studied by using an oxidative stability instrument (OSI-6; Omnion Scientific Instrument). The oxidation cell containing 3 grams of biodiesel sample was heated and kept at 110 ºC and constant 10 L/h airflow was purged through the sample. The easily volatile products generated from biodiesel oxidation could be swept into measuring cell containing 50 mL deionized water and a conductivity probe via air bubbling. The oil stability index (OSI) was determined as the time at which the deionized water conductivity increased sharply.

PV was also used to access the stability of biodiesel after ageing. The determination of biodiesel PV at current study was obtained by conducting following steps:

(1) Ammonium thiocyanate solution (30 % w/v) was prepared by mixing 30 g ammonium thiocyanate at 100 ml volumetric flask and 0.4 M hydrochloride (HCl) was prepared by mixing 40 ml 1 N HCl with 60 ml distilled water.

(2) Preparation of ferrous solution: 0.2 g BaCl2 was added into 25 ml 0.4 M HCl and completed dissolved; then 0.25 g FeSO4.7 H2O was added to 25 ml distilled water and thoroughly mixed as well; these two solutions were mixed at the ratio of 1:1 and then centrifuged at 5000 rpm for 5 min and the final upper layer was ferrous solution.

(3) Generally, 30 µl biodiesel sample was pipetted out to culture glass tube containing 270 ul isooctane: isopropanol mixture (1:1), but the proper dilution of samples might be required in some cases because of extremely high PV of samples and the detection limit of the spectrographic instrument. 2.8 ml methanol: butanol mixture (2:1) was then added into glass culture tube followed by addition of 30 µl ferrous solution: thiocyanate mixture (1:1).

(4) The absorbance of this completely mixed solution (typically purple color) was measured at 510 nm after 20 min. The concentration of PV could be calculated by

computing the obtained absorbance against valid cumene hydroperoxides calibration curve.

6.3.4 Methods of Accelerated Ageing

In order to assess the storage stability of antioxidant treated camelina biodiesel, two different accelerated ageing methods (ageing for 24h and ASTM D4625) were applied in the present study. For the 24h ageing, the modified oxidation cell was present in Fig. 6.2 and the ageing conditions are: 3 grams camelina biodiesel sample was aged in the modified oxidation cell for 24h at 80 ºC with 10 L/h constant airflow, and the exhausts were directly discharged into open air via the outlet pipe without connecting to the original conductivity detection cell. Four replications have been conducted herein and both OSI and PV values of samples were recorded.

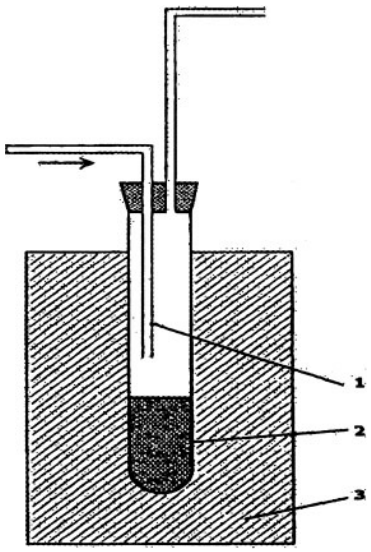


Fig. 6.2 Modified oxidation tube in oxidative stability instrument: (1) Air inlet pipe, positioned at 2.5 cm above the surface of the sample; (2) 3 grams of biodiesel sample; (3) Heating block, enable to maintain the temperature at 80 ºC (± 0.1 ºC). (Adapted from Bondioli et al., 2004 with copyright permission from John Wiley and Sons).

The ageing conditions for camelina biodiesel following ASMT method D4625 are: 400 ml camelina biodiesel sample was placed in 500 ml glass bottle with a lid and held in an oven at 43 ºC to simulate ageing for 12 weeks. One week ageing under these conditions was considered to be equivalent to one month of normal storage at ambient temperature.

Measurements of OSI and PV of aged samples (containing three replication groups) were taken at regular intervals of every two weeks.

6.3.5 Data analysis

The oxidation stability data and the storage stability data obtained after 24h ageing were analyzed by using group comparison method at Minitab 17 while the storage stability data obtained from ASTM D4625 ageing method were analyzed by using repeated measurement method at SAS 9.4. Letter groupings that do not share the same letter indicate significant difference ($p<0.05$).

6.4 Results and Discussion

6.4.1 Effect of Antioxidants on Oxidation Stability of Camelina Biodiesel

Camelina biodiesel is extremely susceptible to oxidative degradation mainly due to its high percent of unsaturated methyl esters, including 14.4 wt.% of methyl oleate (C18:1), 19.1 wt.% of methyl linoleate (C18:2) and 33.5 wt.% of methyl linolenate (C18:3). Polyunsaturated methyl esters were generally much more reactive than the monounsaturated methyl esters (Dunn, 2002). For instance, the relative degradation rate was 98:41:1 for methyl linolenate (C18:3), methyl linoleate (C18:2) and methyl oleate (C18:1) respectively (Frankel, 1980). Many other studies have also illustrated the poor oxidation stability of camelina biodiesel, such as 1.3h (Ciubota-Rosie et al., 2013), 0.6h (Soriano Jr and Narani, 2011) and 2.5h (Moser and Vaughn, 2010) at 110 ºC, lower than the value of 3 hours in the ASTM D6751 and 8 hours in EN14214:2014.

Treating biodiesel with synthetic antioxidants is an effective way to stabilize FAME, therefore, increase the resistance to oxidative degradation. In this study, the parameter OSI was measured to assess the oxidation stability of camelina biodiesel treated with BHA, BHT, PrG and TBHQ at different concentrations as shown in Fig. 6.3. The stability of the camelina biodiesel treated with BHA was not satisfactory, in the concentration range of 0 ppm to 3000 ppm, the oxidation stability were lower than the limit of 8 hours, and therefore failed to meet the requirements in EN14214. In addition, it was observed that increasing the BHA dosage did not significantly improve the OSI values.

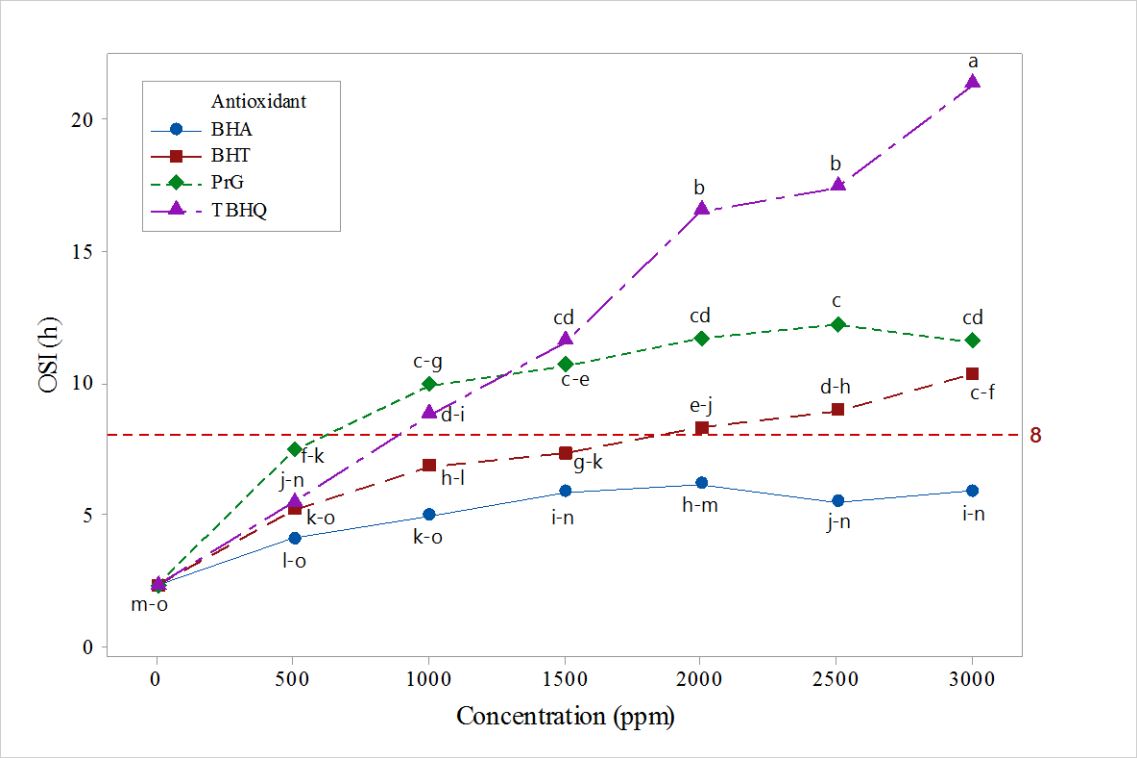


Fig. 6.3 The relationship of Oil Stability Index (OSI) of camelina biodiesel and concentrations of antioxidant (BHA, BHT, PrG and TBHQ).

BHT was a better antioxidant. Adding 1000 ppm BHT into camelina biodiesel increased the OSI from 3h to 6.9h. 2000 ppm BHT was sufficient to raise the OSI to 8.3h. A further increase in the BHT dosage did not greatly increase OSI values.

PrG was observed to be more effective than BHA and BHT as the OSI of camelina biodiesel reached 7.5h when only 500 ppm of PrG was added. Moreover, the OSI of camelina biodiesel treated by PrG was much higher (9.9h > 6.9h) than that of BHT when the level of antioxidants was at 1000 ppm. Surprisingly, it was found that raising PrG dosage from 1000 ppm to 3000 ppm, no significant improvement in the oxidation stability of biodiesel was observed.

Similarly to PrG, a small amount of TBHQ (500 ppm) was able to increase camelina biodiesel oxidation stability from 2.3h to 5.5h. Further increasing TBHQ dosage to 1000 ppm did significantly enhanced OSI to 8.8h, which is satisfactory for EN 14214:2014 specification. Different from the other three antioxidants used in the present study, a noticeably increasing trend of camelina biodiesel OSI was observed by continuously

increasing TBHQ loading, especially for 3000 ppm TBHQ loading the OSI was as high as 21.3h.

From these results, it was found that TBHQ presented the highest antioxidant activity among the four synthetic antioxidants of our research interest, and the order of effectiveness in improving the oxidation stability of camelina biodiesel, were TBHQ > PrG > BHT > BHA. This was in agreement with results reported in many other studies (Domingos et al., 2007; Mittelbach and Schober, 2003; Tang et al., 2008). For instance, Mittelbach and Schober, (2003) has shown that TBHQ and PrG at 1000 ppm were more effective at promoting oxidation stability of biodiesel derived from rapeseed oil, used frying oil and tallow compared to BHT and BHA. Domingos et al., (2007) stated that increasing BHA concentration (from 2000 ppm to 8000 ppm) did not provide noticeable enhancement on biodiesel induction time and TBHQ displayed a great stabilizing potential for soybean ethyl esters when used at a high loading concentration (up to 8000 ppm). Tang et al., (2008) demonstrated that the effect of each antioxidant on the stability of biodiesel varied with the original feedstock oils; PrG and TBHQ (250 -1000 ppm) can significantly improve the induction period of biodiesel derived from soybean, cottonseed and yellow grease, however, BHA and BHT showed the best results for biodiesel made from poultry fat.

In the current study, the oxidation stability (OSI) of camelina biodiesel was improved to a satisfactory level by adding either 2000 ppm BHT, 1000 ppm PrG or 1000 ppm TBHQ. Therefore, in the following section of studying the storage stability, BHT, PrG and TBHQ were used and the antioxidant dosage range was set from 1000 ppm to 3000 ppm.

6.4.2 Effect of Antioxidants on Storage Stability of Camelina Biodiesel Aged for 24h

Newly released EN 14214:2014 requires a minimum OSI of 8h to ensure adequate biodiesel stability during a typical 6-month fuel consumption timeframe (Christensen and McCormick, 2014). However, there is no specific requirement for biodiesel storage stability. This is mainly due to the storage stability could be greatly influenced by its storage environment which varied with different manufacturers. Nevertheless, it is critically important for biodiesel producers as well as customers to monitor and predict biodiesel quality over the storage period, which might be longer than 6 months. A reliable

and accelerated ageing method used for assessing biodiesel quality is very necessary in this regard. In Europe, under the financial support of BIOSTAT-Stability of Biodiesel project, Bondioli et al. (2004) developed an accelerated ageing method for biodiesel storage stability prediction. They suggested that a 24 hours ageing at 80°C could predict one-year ageing in a reasonably accurate way.

In this study, this newly developed ageing method was used to study the stability of camelina biodiesel treated with different levels of antioxidants after one-year storage. According to the experimental results obtained from section 6.4.1, three antioxidants (TBHQ, PrG and BHT) that gave relatively better results of improving camelina biodiesel oxidation stability were selected for further studies. Their concentrations were set in the range of 1000 ppm to 3000 ppm. The relationships between OSI of camelina biodiesel after 24h ageing and antioxidant concentrations were presented in Fig. 6.4.

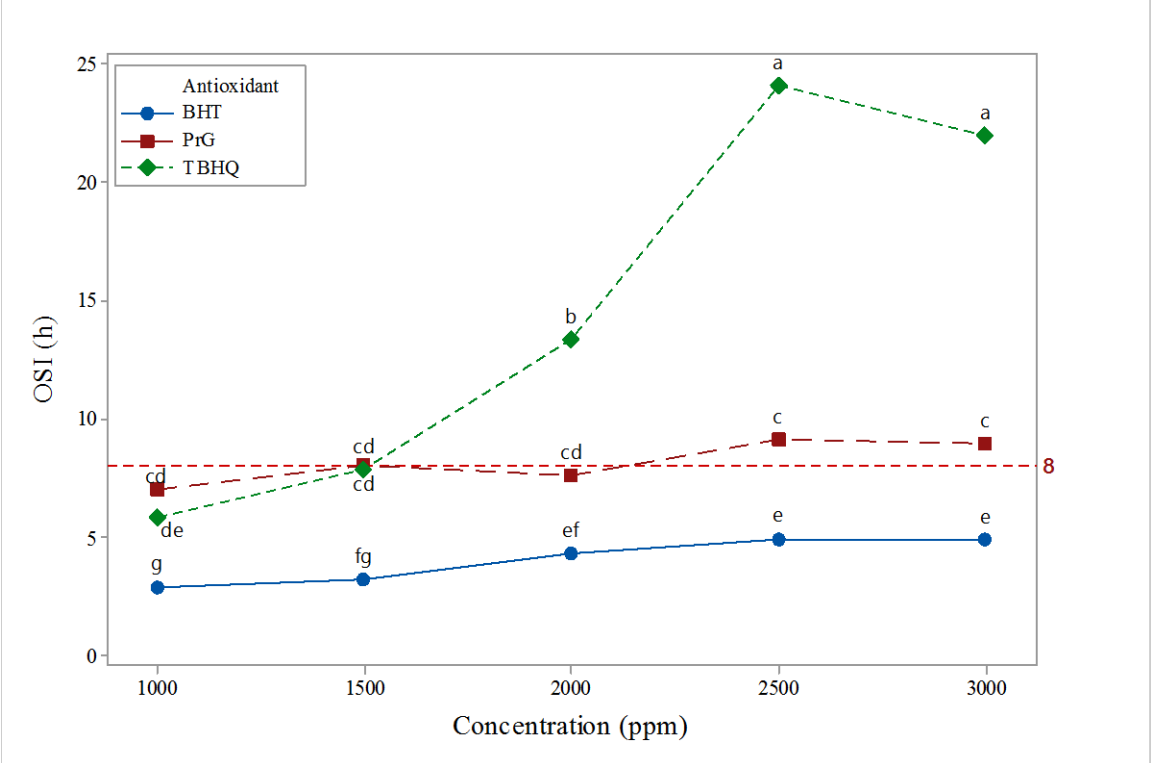


Fig. 6.4 The relationship between Oil Stability Index (OSI) of camelina biodiesel after 24h ageing and concentrations of antioxidant (BHT, PrG and TBHQ).

The OSI of BHT treated camelina biodiesel after 24h ageing, a general prediction of biodiesel OSI for one-year real storage, was less than 8h at all levels in this study. This implied that treating camelina biodiesel with a high concentration (3000 ppm) of BHT can

improve its OSI to a satisfactory level (8h), however, its OSI would decrease to as low as 4.9h (< 8h) after one-year storage. Therefore, BHT was not considered as an effective antioxidant to stabilize camelina biodiesel for one-year storage. Camelina biodiesel samples treated with PrG retained satisfactory OSI (around 8h) after 24h ageing, and no significant change of OSI was observed by increasing PrG concentration. PrG was capable to maintain camelina biodiesel stability around 8h at all levels after one-year storage. In contrast, the stability of camelina biodiesel treated by TBHQ varied greatly with TBHQ's concentrations. OSI values were 13.3h (2000 ppm TBHQ), 7.8h (1500 ppm TBHQ) and 5.8h (1000 ppm TBHQ) respectively. Significantly high OSI was achieved at 2500 ppm TBHQ (24h) as shown in Fig. 6.4. High concentration of TBHQ was extremely effective to increase the resistance of camelina biodiesel against external environment during storage.

PV of antioxidant treated camelina biodiesel after 24h ageing was also measured to assess its storage stability in this study. Fig. 6.5 showed the relationship between PV of the aged camelina biodiesel and antioxidant concentrations. As observed, the PV values were in the range of 15-19 mM and did not change much with the concentrations of BHT. PrG showed the similar trend with PV values ranging from 12 mM to 16 mM. Interestingly, the OSI values of both BHT and PrG treated camelina biodiesel after 24h ageing did not vary significantly with their loading concentrations.

As for TBHQ, the addition of this antioxidant led to an extremely low PV value (only about 5 mM), indicating that TBHQ had higher antioxidant activity than BHT and PrG for a long period of storage. Surprisingly, there was no significant difference in PV of camelina biodiesel treated with 1000 ppm (5.3 mM) and 2000 ppm (4.8 mM) TBHQ, however, their OSI was significantly different (5.8h and 13.4h for 1000 ppm and 2000 ppm TBHQ respectively). This might be referred to that adding a relatively less amount of TBHQ (1000 ppm) into camelina biodiesel can eliminate its hydroperoxide formation during ageing period. The secondary oxidation products (such as easily volatile acids) were formed quickly at 24h aged camelina biodiesel that was treated with relatively low TBHQ loading, and then resulted in relatively low OSI even though just small amounts of hydroperoxides were generated (low PV). This can also be explained that more volatile components were formed (such as crotonaldehyde) during the oxidation of C18:3 as opposed to longer and

less volatile components in the oxidation of C18:2 (Frankel, 1980). Chuck et al., (2012) also reported that different with C18:2, the decomposition rate of C18:3 was highest at the beginning of reaction even though both of them were found to be decomposed almost instantaneously under reaction condition (at 110 ºC).

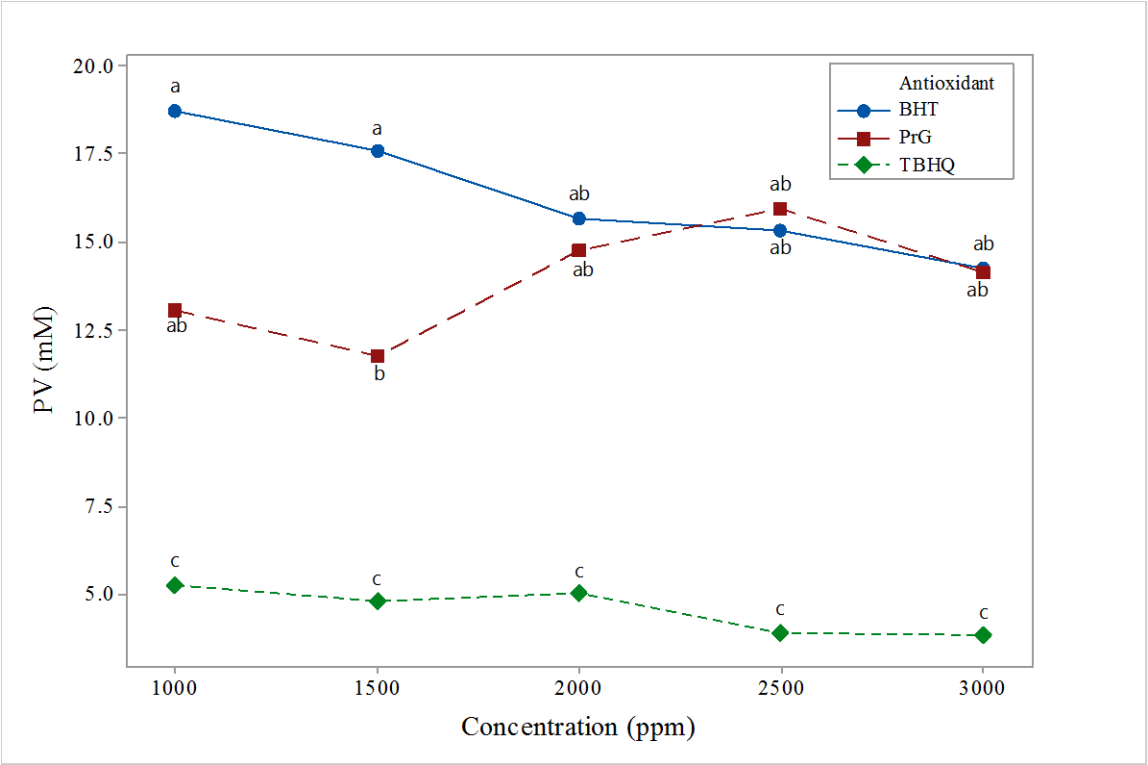


Fig. 6.5 The relationship between peroxide value (PV) of camelina biodiesel after 24h ageing and concentrations of antioxidant (BHT, PrG and TBHQ).

Based on the experimental results, the order of effectiveness of antioxidant on stabilizing camelina biodiesel after one-year storage was: TBHQ > PrG > BHT. However, BHT was not able to maintain 8h OSI of camelina biodiesel stored for one year. Treating camelina biodiesel with 1500 ppm PrG or TBHQ can ensure satisfactory OSI after one-year storage at an ambient temperature based on accelerated 24h ageing method proposed by Bondioli et al., (2004).

6.4.3 Effect of Antioxidants on Storage Stability of Camelina Biodiesel Aged by the ASTM D4625

Improvement of biodiesel storage stability allows it to be kept for a longer duration of time without fear of severe degradation and formation of oxidation products which can

either cause engine damage or lower its fuel qualities (Osawa et al., 2015). Adding antioxidants to biodiesel can delay the oxidation initiation, therefore, increase the induction time until the antioxidant is exhausted, but could not permanently prevent the biodiesel from autoxidation during a long term storage period (Dunn, 2005). To our knowledge, no published literature has studied biodiesel storage stability by using 24h accelerated ageing method yet and ASTM method D4625 (Diesel fuel storage stability at 43 ºC) was usually considered as a promising accelerated ageing method for research on the storage properties of biodiesel (Bondioli et al., 2002; Christensen and McCormick, 2014). A week of 43 ºC storage is roughly equivalent to a month of storage at normal (environmental) ambient temperature. OSI and PV could be measured to monitor degradation of biodiesel aged under storage conditions simulating ASTM D4625 (Bondioli et al., 2004, 2002; R. Dunn, 2005). The relationship between OSI of antioxidant treated camelina biodiesel and ageing weeks at different concentrations were shown in Fig. 6.6.

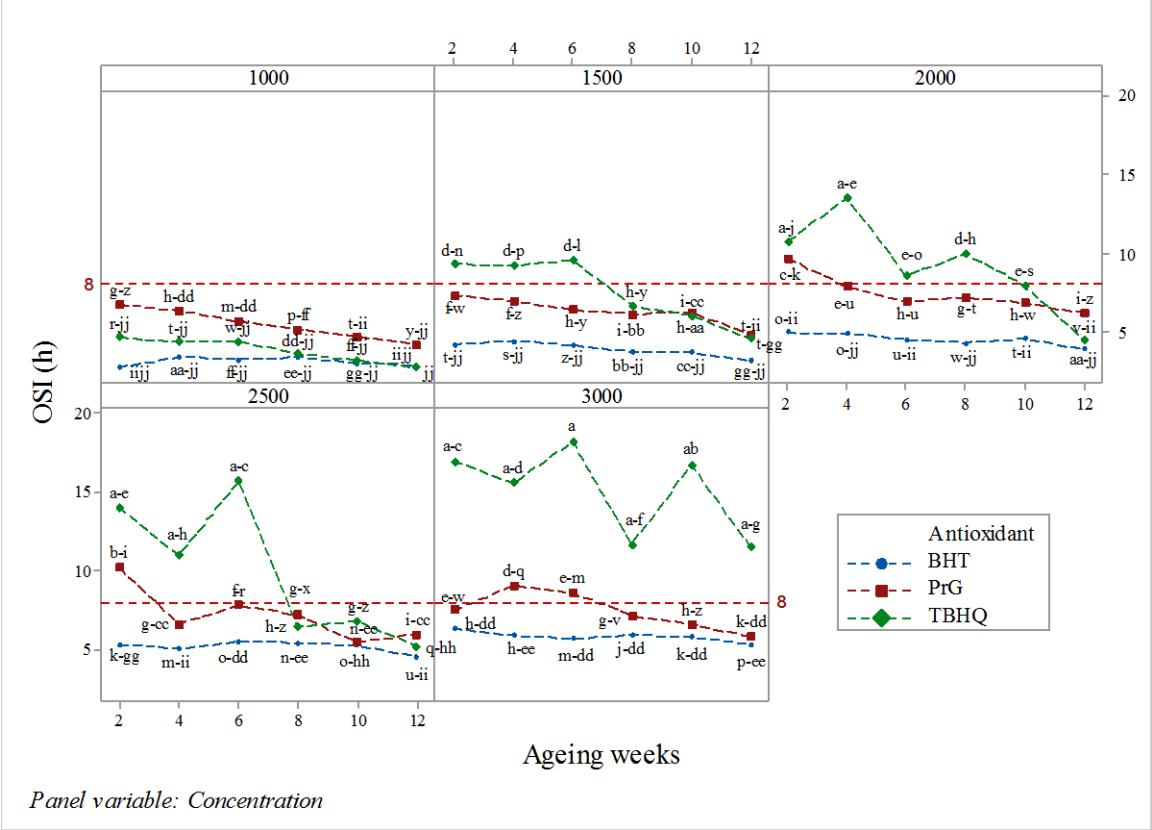


Fig. 6.6 The relationship between Oil Stability Index (OSI) of camelina biodiesel aged by ASTM D4625 and concentrations of antioxidant (BHT, PrG and TBHQ).

The OSI of camelina biodiesel treated with BHT at all levels were all less than 8h throughout 12 weeks ageing process. This result is consistent with the BHT result obtained using the 24h accelerated method and therefore further confirmed that BHT was not an effective antioxidant for camelina biodiesel for a long period of storage. The antioxidant activity of PrG was better than BHT, however, most of the OSI of PrG treated camelina biodiesel were less than 8h. This was different from the results of the experiment using 24h ageing, in which PrG at all concentrations were effective for stabilizing camelina biodiesel over one-year storage.

1000 ppm TBHQ was not sufficient to keep camelina biodiesel OSI at the satisfactory level even at the very beginning of ageing process (such as week 2 and week 4). However, increasing TBHQ loading to 1500 ppm significantly improved camelina biodiesel OSI to 9.3h in week 2 and maintain the acceptable stability until week 6. The stability of camelina biodiesel treated with 2000 ppm TBHQ sustained to week 10. A significant decline of OSI (from 15.7h to 6.7h) of camelina biodiesel treated with 2500 ppm TBHQ was observed after 6 weeks aging and stayed lower than 8h in the following ageing process. Treating camelina biodiesel with 3000 ppm TBHQ exhibited the highest antioxidant effectiveness over the 12 weeks ageing period even though no significant decrease of OSI was detected along with increasing ageing time. These results related to TBHQ herein were not completely consistent with resulted obtained in the Section 6.4.2. For example, 1500-2500 ppm TBHQ could not maintain camelina biodiesel at a satisfactory stability level after 12 weeks ageing. PrG presented a similar trend as TBHQ when comparing the OSI values obtained from using these two different aging methods. Combined all the facts, the ASTM D4625 ageing method generated a harsher condition and simulated a more intensive degradation environment than the 24h ageing method suggested by Bondioli et al. (2004).

Fig. 6.7 demonstrated the relationship between PV of antioxidant treated camelina biodiesel and the number of ageing weeks at five different loading concentrations. The noticeable increasing trend was presented for all three kinds of antioxidants (BHT, PrG and TBHQ) at all levels over 12 weeks ageing period. Camelina biodiesel treated with TBHQ was found to have the lowest PV compared to BHT and PrG. This can be well explained by a high antioxidant activity and effectiveness of TBHQ, which eliminated hydroperoxide formation during ageing period. Interestingly, PV of PrG treated camelina

biodiesel aged by ASTM D4625 was higher than that of BHT treated camelina biodiesel at all five antioxidant loading levels. However, there was no significant difference in PV between PrG and BHT treated camelina biodiesel aged for 24h.

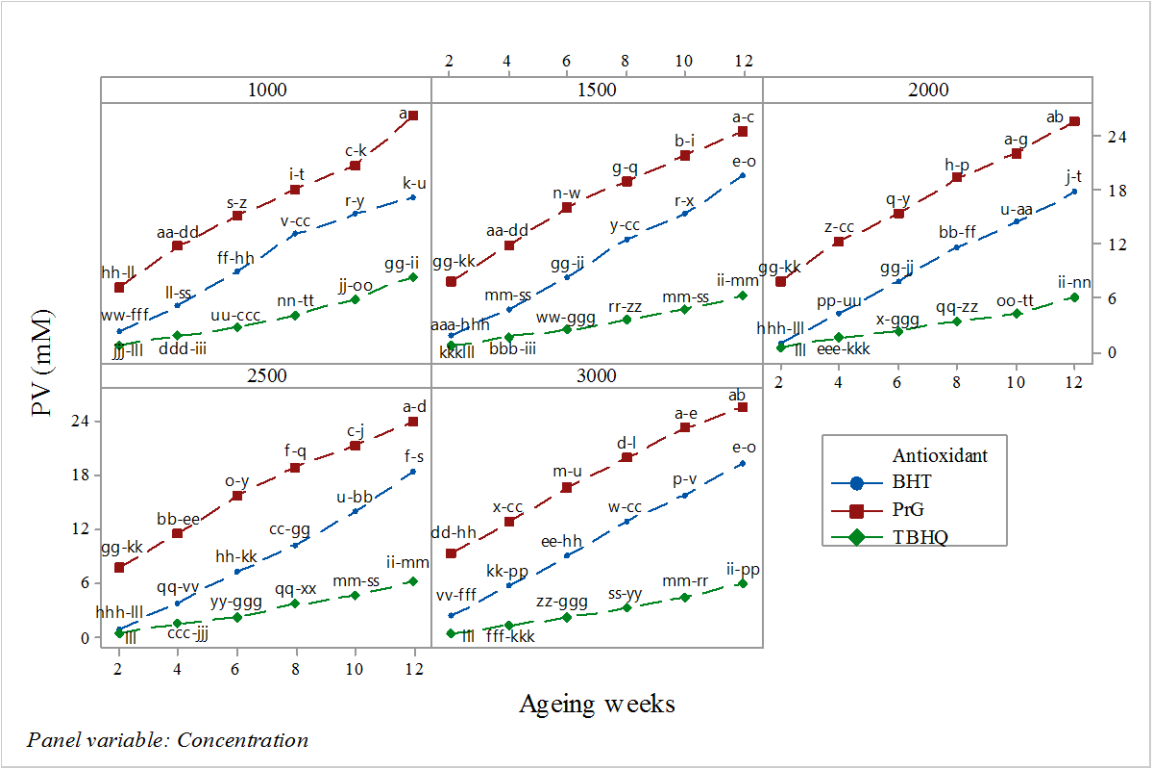


Fig. 6.7 The relationship between peroxide value (PV) of camelina biodiesel aged by ASTM D4625 and concentrations of antioxidant (BHT, PrG and TBHQ).

Synthetic antioxidants containing hydroxyl groups is known to provide protons for free radicals of methyl esters to terminate the free radical chain reaction and form relatively stable radicals. The number of hydroxyl groups within each antioxidant can be ranked as: BHA=BHT (1) < TBHQ (2) < PrG (3) (Tang et al., 2008) as shown in Fig. 6.1. Theoretically, PrG has three hydroxyl groups and can provide more sites for free radicals than others, therefore, PrG should exhibit the highest antioxidant activity. However, PrG had some physical compatibility problems with biodiesel in this study and this was observed by Dunn (Dunn, 2005) as well. A trace amount of PrG sediment was observed at the bottom of the glass bottle during storage in the present study even though it was completely dissolved in biodiesel in the sample preparation processes. Therefore, the antioxidant activity of PrG might be slightly decreased due to its uneven attribution in the biodiesel sample. This might be the reason for a higher PV value of PrG treated camelina

biodiesel aged by the ASTM D4625 as compared to BHT. Additionally, antioxidant activity against oxidative degradation involved very complex processes and also varied with different feedstock oils (Supriyono et al., 2015; Zuleta et al., 2012; Kurechi et al., 1983). Many studies have demonstrated that TBHQ had higher antioxidant activity than PrG including current study. Kurechi et al., (1983) also reported that TBHQ may generate other identical oxidation products during its antioxidative process, which had higher antioxidative activity than TBHQ itself. This can be attributed to the high antioxidant activity and effectiveness of TBHQ. Overall, only TBHQ at a concentration level of 3000 ppm enabled to stabilize camelina biodiesel over the whole 12 weeks of ageing period and TBHQ in the concentration range of 1500-2500 ppm was sufficient to maintain camelina biodiesel stability above 8h for 6 weeks using ASTM D4625 ageing methods.

6.5 Conclusions

Treating biodiesel with an oxidation inhibitor (antioxidant) is a commonly used and promising approach to increase the resistance against biodiesel oxidative degradation. Biodiesel derived from *Camelina sativa* oil has poor oxidative stability. Among four synthetic antioxidants tested in this study, TBHQ presented the highest anti-oxidation effectiveness on improving camelina biodiesel oxidative stability. The oxidation stability index (OSI) of camelina biodiesel was increased from originally lower than 3h to the satisfactory level of 8h by adding either 2000 ppm BHT, 1000 ppm PrG or 1000 pm TBHQ.

The storage stability of camelina biodiesel was assessed as well through measuring parameters OSI and PV using two different ageing methods, namely, 24h accelerated ageing method and the ASTM D4625 ageing method. Ageing camelina biodiesel by the ASTM D4625 simulated more intensive degradation of camelina biodiesel compared to the 24h ageing method. 3000 ppm TBHQ was sufficient to stabilize camelina biodiesel for an ageing period of 12 weeks (equivalent to one-year storage) based on the ASTM D4625 ageing method. Treating camelina biodiesel with only 1500 ppm PrG or TBHQ can obtain the satisfactory value of OSI after one-year storage based on the accelerated 24 h ageing method.

6.6 Acknowledgement

The authors acknowledge the financial support from the Department of Agriculture, Nova Scotia, through Growing Forward 2 Research Acceleration Program. The authors are also grateful to Yougui Chen and Dr. Haibo Niu for the technical assistance of PV determination.

CHAPTER 7: OVERALL CONCLUSIONS AND FUTURE WORK

7.1 Overall Conclusions

Camelina sativa has been recognized as a promising energy crop for biodiesel production in North America. The research presented herein has demonstrated that it is feasible to produce biodiesel from *Camelina sativa* grown in Nova Scotia. To assess its feasibility, the fatty acid profile of camelina oil, fuel properties of camelina biodiesel, alkali-catalyzed transesterification process, crude camelina biodiesel purification and the oxidative stability of camelina biodiesel were investigated.

- The fatty acid profile of *camelina sativa* oil was identified to contain 10%, 33.2% and 56.8% saturated, monounsaturated and polyunsaturated fatty acids respectively, especially, with high percentages of linolenic acid (C18:3; 33..5 wt. %) and linoleic acid (C18:2; 19.1 wt. %).
- An alkali-catalyzed transesterification process can readily convert the parent *camelina sativa* oil into camelina biodiesel. The RSM method was used to investigate the effects of four independent factors (temperature, time, molar ratio of methanol/oil, and catalyst concentration) on the dependent variables (product yield and FAME yield) and to further optimize the alkali-catalyzed transesterification conditions. The optimal conditions were determined to be the reaction temperature of 38.7°C, 40 min of reaction time, 7.7 of molar ratio of methanol/oil, and 1.50 wt. % of catalyst concentration. At such optimal conditions, the maximum camelina biodiesel product yield of 97% and FAME yield of 98.9% were obtained.
- It is also demonstrated that a dry washing process using fiber-based biosorbents is an alternative option for biodiesel purification. The soap removal capacity of three studied biosorbents are 9.4 mL/g for wood shavings, 24.4 mL/g for sawdust and 51.1 mL/g for the commercially available BD-Zorb respectively, when the initial soap content of the crude camelina biodiesel was 9007 ppm. In comparison with the other purification method, wet washing, a large amount of distilled water (14:1 of water/biodiesel in volume at 50 ºC and 30:1 water/biodiesel in volume at 20 ºC)

was needed to reduce the same soap content in camelina biodiesel to a satisfactory level.

- Most of the fuel properties of the resulting camelina biodiesel were in good agreement with the specifications in the ASTM D6751 and EN 14214, including kinematic viscosity, acid number, flash point, sulfur content, total glycerol content and mono-, di-and triglycerides. Its cetane number (49.7) was satisfactory according to the ASTM D6751, but not for the EN 14214. However, camelina biodiesel exhibited poor oxidative stability (1.9 h), resulting from a high percentage of polyunsaturated fatty acid methyl esters.
- The addition of synthetic antioxidants can effectively increase biodiesel's resistance against auto-oxidative degradation. The oxidation stability (OSI) of camelina biodiesel was improved from originally lower than 3h to a satisfactory level (8h) by adding either 2000 ppm BHT, 1000 ppm PrG or 1000 pm TBHQ. The storage stability of camelina biodiesel was assessed by using two different ageing methods, namely 24h accelerated ageing and the ageing method described in the ASTM D4625. 3000 ppm TBHQ was sufficient to stabilize camelina biodiesel within 12 weeks ageing period (equivalent to one-year storage) based on the ASTM D4625 ageing method. Treating camelina biodiesel with 1500 ppm PrG or TBHQ can ensure satisfactory stability for one-year storage based on the accelerated 24h ageing method. In the scope of this study, TBHQ presented the highest antioxidant effectiveness on improving camelina biodiesel oxidative stability, and both the oxidation and storage stability of camelina biodiesel were improved to a satisfactory level by adding a suitable amount of TBHQ.

7.2 Future Work

The feasibility of biodiesel production from *Camelina sativa* oil has been demonstrated in the present study. However, more research related to fuel properties improvement, by-product utilization, the application of heterogeneous catalyst and scaling up issues, are essential to be further conducted to increase the overall economic viability of camelina biodiesel production.

Generally speaking, all types of biodiesel exhibit poor cold flowability compared to that of petrodiesel. The cold flow properties of camelina biodiesel, therefore, need to be further investigated. The utilization of the by-product, glycerol is critical for reducing biodiesel production costs. Attempts of using glycerol as a binding agent for solid biofuels (such as pellets and briquettes) should be made in future work. Producing camelina biodiesel in the presence of heterogeneous catalysts should be explored as the heterogeneous catalysts can be easily separated from product mixture, and thus significantly reduce the burden to purification steps.

The study on scaling up of camelina biodiesel production under the optimal reaction conditions obtained from this study would be necessary for a pilot production in the near future. The introduction of ultrasound or microwave-assisted biodiesel production is promising and deserves more attention.

REFERENCES

Abdulla, R., Ravindra, P., 2013. Immobilized Burkholderia cepacia lipase for biodiesel production from crude Jatropha curcas L. oil. Biomass Bioenergy 56, 8–13.

Anwar, F., Rashid, U., Ashraf, M., Nadeem, M., 2010. Okra (Hibiscus esculentus) seed oil for biodiesel production. Appl. Energy 87, 779–785.

Atabani, A.E., Silitonga, A.S., Badruddin, I.A., Mahlia, T.M.I., Masjuki, H.H., Mekhilef, S., 2012. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. Renew. Sustain. Energy Rev. 16, 2070–2093.

Atadashi, I.M., 2015. Purification of crude biodiesel using dry washing and membrane technologies. Alex. Eng. J. 54, 1265–1272.

Atadashi, I.M., Aroua, M.K., Abdul Aziz, A.R., Sulaiman, N.M.N., 2013. The effects of catalysts in biodiesel production: A review. J. Ind. Eng. Chem. 19, 14–26.

Atadashi, I.M., Aroua, M.K., Aziz, A.A., 2011. Biodiesel separation and purification: A review. Renew. Energy 36, 437–443.

Atadashi, I.M., Aroua, M.K., Aziz, A.R.A., Sulaiman, N.M.N., 2011. Refining technologies for the purification of crude biodiesel. Appl. Energy 88, 4239–4251.

Awad, S., Paraschiv, M., Varuvel, E.G., Tazerout, M., 2013. Optimization of biodiesel production from animal fat residue in wastewater using response surface methodology. Bioresour. Technol. 129, 315–320.

Banković-Ilić, I.B., Stamenković, O.S., Veljković, V.B., 2012. Biodiesel production from non-edible plant oils. Renew. Sustain. Energy Rev. 16, 3621–3647.

Bautista, L.F., Vicente, G., Rodríguez, R., Pacheco, M., 2009. Optimisation of FAME production from waste cooking oil for biodiesel use. Biomass Bioenergy 33, 862–872.

Berrios, M., Skelton, R.L., 2008. Comparison of purification methods for biodiesel. Chem. Eng. J. 144, 459–465.

Bondioli, P., Gasparoli, A., Della Bella, L., Tagliabue, S., 2002. Evaluation of biodiesel storage stability using reference methods. Eur. J. Lipid Sci. Technol. 104, 777–784.

Bondioli, P., Gasparoli, A., della Bella, L., Tagliabue, S., Lacoste, F., Lagardere, L., 2004. The prediction of biodiesel storage stability. Proposal for a quick test. Eur. J. Lipid Sci. Technol. 106, 822–830.

Bondioli, P., Gasparoli, A., Lanzani, A., Fedeli, E., Veronese, S., Sala, M., 1995. Storage stability of biodiesel. J. Am. Oil Chem. Soc. 72, 699–702.

Bouaid, A., Martinez, M., Aracil, J., 2007. Long storage stability of biodiesel from vegetable and used frying oils. Fuel 86, 2596–2602.

Box, G.E.P., Draper, N.R., 1987. Empirical model-building and response surfaces. Wiley.

Canakci, M., Monyem, A., Van Gerpen, J., 1999. Accelerated Oxidation Processes Is Biodiesel. Trans. ASAE 42.

Cao, L., Zhang, S., 2015. Production and characterization of biodiesel derived from Hodgsonia macrocarpa seed oil. Appl. Energy 146, 135–140.

Ceylan, H., Kubilay, S., Aktas, N., Sahiner, N., 2008. An approach for prediction of optimum reaction conditions for laccase-catalyzed bio-transformation of 1-naphthol by response surface methodology (RSM). Bioresour. Technol. 99, 2025–2031.

Chakraborty, M., Baruah, D.C., 2013. Production and characterization of biodiesel obtained fromSapindus mukorossikernel oil. Energy 60, 159–167.

Christensen, E., McCormick, R.L., 2014. Long-term storage stability of biodiesel and biodiesel blends. Fuel Process. Technol. 128, 339–348.

Chuck, C.J., Jenkins, R.W., Bannister, C.D., Han, L., Lowe, J.P., 2012. Design and preliminary results of an NMR tube reactor to study the oxidative degradation of fatty acid methyl ester. Biomass Bioenergy 47, 188–194.

Ciubota-Rosie, C., Ruiz, J.R., Ramos, M.J., Pérez, Á., 2013a. Biodiesel from Camelina sativa: A comprehensive characterisation. Fuel 105, 572–577.

Demirbas, A., 2011. Competitive liquid biofuels from biomass. Appl. Energy 88, 17–28.

Domingos, A.K., Saad, E.B., Vechiatto, W.W.D., Wilhelm, H.M., Ramos, L.P., 2007. The influence of BHA, BHT and TBHQ on the oxidation stability of soybean oil ethyl esters (biodiesel). J. Braz. Chem. Soc. 18, 416–423.

Dunn, R.O., 2005. Oxidative Stability of Soybean Oil Fatty Acid Methyl Esters by Oil Stability Index (OSI). JAOCS J. Am. Oil Chem. Soc. 82, 381–387.

Dunn, R.O., 2009. Effects of minor constituents on cold flow properties and performance of biodiesel. Prog. Energy Combust. Sci. 35, 481–489.

Dunn, R.O., 2008. Antioxidants for improving storage stability of biodiesel. Biofuels Bioprod. Biorefining 2, 304–318.

Dunn, R.O., 2005. Effect of antioxidants on the oxidative stability of methyl soyate (biodiesel). Fuel Process. Technol., Biodiesel Processing and Production 86, 1071–1085.

Dunn, R.O., 2002. Effect of oxidation under accelerated conditions on fuel properties of methyl soyate (biodiesel). J. Am. Oil Chem. Soc. 79, 915–920.

Ernesto C. Zuleta, Libia M Baena, Luis A. Rios, Jorge Calderón, 2012. The oxidative stability of biodiesel and its impact on the deterioration of metallic and polymeric materials: A review. J. Braz. Chem. Soc. 23, 2159–2175.

Faccini, C.S., Cunha, M.E. da, Moraes, M.S.A., Krause, L.C., Manique, M.C., Rodrigues, M.R.A., Benvenutti, E.V., Caramão, E.B., 2011. Dry washing in biodiesel purification: a comparative study of adsorbents. J. Braz. Chem. Soc. 22, 558–563.

Fadhil, A.B., Dheyab, M.M., Abdul-Qader, A.-Q.Y., 2012. Purification of biodiesel using activated carbons produced from spent tea waste. J. Assoc. Arab Univ. Basic Appl. Sci. 11, 45–49.

Fan, X., Chen, F., Wang, X., 2010. Ultrasound-assisted Synthesis of Biodiesel from Crude Cottonseed Oil Using Response Surface Methodology. J. Oleo Sci. 59, 235–241.

Fernández, C.M., Ramos, M.J., Pérez, Á., Rodríguez, J.F., 2010. Production of biodiesel from winery waste: Extraction, refining and transesterification of grape seed oil. Bioresour. Technol. 101, 7019–7024.

Frankel, E.N., 1980. N. Lipid Oxidation. Prog. Lipid Res. 19, 1–22.

Fröhlich, A., Rice, B., 2005. Evaluation of Camelina sativa oil as a feedstock for biodiesel production. Ind. Crops Prod. 21, 25–31.

Gerpen, J.V., Hammond, E.G., Yu, L., Monyem, A., 1997. Determining the influence of contaminants on biodiesel properties. SAE Pap. 971685.

Gerpen, J.V., 2005. Biodiesel processing and production. Fuel Process. Technol. 86, 1097–1107.

Gomes, M.C.S., Arroyo, P.A., Pereira, N.C., 2013. Influence of acidified water addition on the biodiesel and glycerol separation through membrane technology. J. Membr. Sci. 431, 28–36.

Gonzalo, A., García, M., Luis Sánchez, J., Arauzo, J., Peña, J.Á., 2010. Water Cleaning of Biodiesel. Effect of Catalyst Concentration, Water Amount, and Washing Temperature on Biodiesel Obtained from Rapeseed Oil and Used Oil. Ind. Eng. Chem. Res. 49, 4436–4443.

Graboski, M.S., McCormick, R.L., 1998. Combustion of fat and vegetable oil derived fuels in diesel engines. Prog. Energy Combust. Sci. 24, 125–164.

Grunin, Y.B., Grunin, L.Y., Nikol'skaya, E.A., Talantsev, V.I., Gogelashvili, G.S., 2012. Features of the sorption of water vapor and nitrogen on cellulose. Russ. J. Phys. Chem. A 87, 100–103.

Gugel, R.K., Falk, K.C., 2006. Agronomic and seed quality evaluation of Camelina sativa in western Canada. Can. J. Plant Sci. Rev. Can. Phytotech. 86, 1047–1058.

Gui, M.M., Lee, K.T., Bhatia, S., 2008. Feasibility of edible oil vs. non-edible oil vs. waste edible oil as biodiesel feedstock. Energy 33, 1646–1653.

Heinze, T., Liebert, T., 2001. Unconventional methods in cellulose functionalization. Prog. Polym. Sci. 26, 1689–1762.

Hoed, V.V., Zyaykina, N., Greyt, W.D., Maes, J., Verhé, R., Demeestere, K., 2008. Identification and Occurrence of Steryl Glucosides in Palm and Soy Biodiesel. J. Am. Oil Chem. Soc. 85, 701–709.

Hrastar, R., Petrišič, M.G., Ogrinc, N., Košir, I.J., 2009. Fatty Acid and Stable Carbon Isotope Characterization of Camelina sativa Oil: Implications for Authentication. J. Agric. Food Chem. 57, 579–585.

International Energy Statistics - EIA [WWW Document], n.d. URL http://www.eia.gov/cfapps/ipdbproject/iedindex3.cfm?tid=5&pid=63&aid=2&cid=regions&syid=2010&eyid=2014&unit=TBPD (accessed 3.11.16).

Iskandarov, U., Kim, H.J., Cahoon, E.B., 2014. Camelina: An Emerging Oilseed Platform for Advanced Biofuels and Bio-Based Materials, in: McCann, M.C., Buckeridge, M.S., Carpita, N.C. (Eds.), Plants and BioEnergy, Advances in Plant Biology. Springer New York, pp. 131–140.

Jain, S., Sharma, M.P., 2010. Stability of biodiesel and its blends: A review. Renew. Sustain. Energy Rev. 14, 667–678.

Jaliliannosrati, H., Amin, N.A.S., Talebian-Kiakalaieh, A., Noshadi, I., 2013. Microwave assisted biodiesel production from Jatropha curcas L. seed by two-step in situ process: Optimization using response surface methodology. Bioresour. Technol. 136, 565–573.

Jiang, Y., Caldwell, C.D., Falk, K.C., 2014. Camelina seed quality in response to applied nitrogen, genotype and environment. Can. J. Plant Sci. 94, 971–980. doi:10.4141/cjps2013-396.

Kang, J., Snapp, A.R., Lu, C., 2011. Identification of three genes encoding microsomal oleate desaturases (FAD2) from the oilseed crop Camelina sativa. Plant Physiol. Biochem. 49, 223–229.

Karaosmanoğlu, F., Cığızoğlu, K.B., Tüter, M., Ertekin, S., 1996. Investigation of the Refining Step of Biodiesel Production. Energy Fuels 10, 890–895.

Kirkhus, B., Lundon, A.R., Haugen, J.-E., Vogt, G., Borge, G.I.A., Henriksen, B.I.F., 2013. Effects of Environmental Factors on Edible Oil Quality of Organically Grown Camelina sativa. J. Agric. Food Chem. 61, 3179–3185.

Knothe, G., 2007. Some aspects of biodiesel oxidative stability. Fuel Process. Technol., Biofuels for Transportation 88, 669–677.

Knothe, G., 2006. Analysis of oxidized biodiesel by 1H-NMR and effect of contact area with air. Eur. J. Lipid Sci. Technol. 108, 493–500.

Knothe, G., 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process. Technol., Biodiesel Processing and Production 86, 1059–1070.

Knothe, G., Dunn, R.O., 2003. Dependence of oil stability index of fatty compounds on their structure and concentration and presence of metals. J. Am. Oil Chem. Soc. 80, 1021–1026.

Kouzu, M., Hidaka, J., 2013. Purification to remove leached CaO catalyst from biodiesel with the help of cation-exchange resin. Fuel 105, 318–324.

Krohn, B.J., Fripp, M., 2012. A life cycle assessment of biodiesel derived from the “niche filling” energy crop camelina in the USA. Appl. Energy 92, 92–98.

Kurechi, T., Aizawa, M., Kunugi, A., 1983. Studies on the antioxidants XVIII: Oxidation product of tertiary butyl hydroquinone (TBHQ) (I). J. Am. Oil Chem. Soc. 60, 1878–1882.

Lebedevas, S., Makareviciene, V., Sendzikiene, E., Zaglinskis, J., 2013. Oxidation stability of biofuel containing Camelina sativa oil methyl esters and its impact on energy and environmental indicators of diesel engine. Energy Convers. Manag., Global Conference on Renewable energy and Energy Efficiency for Desert Regions 2011 "GCREEDER 2011" 65, 33–40.

Lee, I., Pfalzgraf, L.M., Poppe, G.B., Powers, E., Haines, T., 2007. The role of sterol glucosides on filter plugging. Biodiesel Mag. 4, 105–112.

Leung, D.Y.C., Guo, Y., 2006. Transesterification of neat and used frying oil: Optimization for biodiesel production. Fuel Process. Technol. 87, 883–890.

Leung, D.Y.C., Wu, X., Leung, M.K.H., 2010. A review on biodiesel production using catalyzed transesterification. Appl. Energy 87, 1083–1095.

Liang, Y.C., May, C.Y., Foon, C.S., Ngan, M.A., Hock, C.C., Basiron, Y., 2006. The effect of natural and synthetic antioxidants on the oxidative stability of palm diesel. Fuel 85, 867–870.

Manique, M.C., Faccini, C.S., Onorevoli, B., Benvenutti, E.V., Caramão, E.B., 2012. Rice husk ash as an adsorbent for purifying biodiesel from waste frying oil. Fuel 92, 56–61.

Marinova, E., Toneva, A., Yanishlieva, N., 2008. Synergistic antioxidant effect of α-tocopherol and myricetin on the autoxidation of triacylglycerols of sunflower oil. Food Chem. 106, 628–633.

Meher, L.C., Churamani, C.P., Arif, M., Ahmed, Z., Naik, S.N., 2013. Jatropha curcas as a renewable source for bio-fuels—A review. Renew. Sustain. Energy Rev. 26, 397–407.

Mittelbach, M., Schober, S., 2003. The influence of antioxidants on the oxidation stability of biodiesel. J. Am. Oil Chem. Soc. 80, 817–823.

Modiba, E., Osifo, P., Rutto, H., 2014. Biodiesel production from baobab (Adansonia digitata L.) seed kernel oil and its fuel properties. Ind. Crops Prod. 59, 50–54.

Mofijur, M., Masjuki, H.H., Kalam, M.A., Hazrat, M.A., Liaquat, A.M., Shahabuddin, M., Varman, M., 2012. Prospects of biodiesel from Jatropha in Malaysia. Renew. Sustain. Energy Rev. 16, 5007–5020.

Monyem, A., H. Van Gerpen, J., 2001. The effect of biodiesel oxidation on engine performance and emissions. Biomass Bioenergy 20, 317–325.

Mootabadi, H., Salamatinia, B., Bhatia, S., Abdullah, A.Z., 2010. Ultrasonic-assisted biodiesel production process from palm oil using alkaline earth metal oxides as the heterogeneous catalysts. Fuel 89, 1818–1825.

Moser, B.R., Vaughn, S.F., 2010. Evaluation of alkyl esters from Camelina sativa oil as biodiesel and as blend components in ultra low-sulfur diesel fuel. Bioresour. Technol. 101, 646–653.

Myers, R.H., Montgomery, D.C., Anderson-Cook, C.M., 2009. Response Surface Methodology: Process and Product Optimization Using Designed Experiments, third ed. ed.

Nigam, P.S., Singh, A., 2011. Production of liquid biofuels from renewable resources. Prog. Energy Combust. Sci. 37, 52–68.

Osawa, W.O., Sahoo, P.K., Onyari, J.M., Mulaa, F.J., 2015. Effects of antioxidants on oxidation and storage stability of Croton megalocarpus biodiesel. Int. J. Energy Environ. Eng. 1–7.

Patil, P.D., Deng, S., 2009. Optimization of biodiesel production from edible and non-edible vegetable oils. Fuel 88, 1302–1306.

Patil, P.D., Gude, V.G., Deng, S., 2009. Biodiesel Production from Jatropha Curcas, Waste Cooking, and Camelina Sativa Oils. Ind. Eng. Chem. Res. 48, 10850–10856.

Plessis, L.M.D., Villiers, J.B.M.D., Walt, W.H.V.D., 1985. Stability studies on methyl and ethyl fatty acid esters of sunflowerseed oil. J. Am. Oil Chem. Soc. 62, 748–752.

Ramírez-Verduzco, L.F., Rodríguez-Rodríguez, J.E., Jaramillo-Jacob, A. del R., 2012. Predicting cetane number, kinematic viscosity, density and higher heating value of biodiesel from its fatty acid methyl ester composition. Fuel 91, 102–111.

Rand, S.J., Westbrook, S.R., LeCren, R.T., 2010. Fuels for Land and Marine Diesel Engines and for Nonaviation Gas Turbines, in: Rand, S.J. (Ed.), Significance of

Tests for Petroleum Products: 8th Edition. ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, pp. 33–33–20.

Rashid, U., Anwar, F., 2008. Production of biodiesel through optimized alkaline-catalyzed transesterification of rapeseed oil. Fuel 87, 265–273.

Rashid, U., Anwar, F., Moser, B.R., Ashraf, S., 2008. Production of sunflower oil methyl esters by optimized alkali-catalyzed methanolysis. Biomass Bioenergy 32, 1202–1205.

Rudnik, E., Szczucinska, A., Gwardiak, H., Szulc, A., Winiarska, A., 2001. Comparative studies of oxidative stability of linseed oil. Thermochim. Acta 370, 135–140.

Saleh, J., Tremblay, A.Y., Dubé, M.A., 2010. Glycerol removal from biodiesel using membrane separation technology. Fuel 89, 2260–2266.

Séguin-Swartz, G., Eynck, C., Gugel, R.K., Strelkov, S.E., Olivier, C.Y., Li, J.L., Klein-Gebbinck, H., Borhan, H., Caldwell, C.D., Falk, K.C., 2009. Diseases of Camelina sativa (false flax). Can. J. Plant Pathol. 31, 375–386.

Serra, T., Zilberman, D., 2013. Biofuel-related price transmission literature: A review. Energy Econ. 37, 141–151.

Soriano Jr, N.U., Narani, A., 2011. Evaluation of Biodiesel Derived from Camelina sativa Oil. J. Am. Oil Chem. Soc. 89, 917–923.

Squissato, A.L., Fernandes, D.M., Sousa, R.M.F., Cunha, R.R., Serqueira, D.S., Richter, E.M., Pasquini, D., Muñoz, R.A.A., 2015. Eucalyptus pulp as an adsorbent for biodiesel purification. Cellulose 22, 1263–1274.

Stojković, I.J., Stamenković, O.S., Povrenović, D.S., Veljković, V.B., 2014. Purification technologies for crude biodiesel obtained by alkali-catalyzed transesterification. Renew. Sustain. Energy Rev. 32, 1–15.

Supriyono, Sulistyo, H., Almeida, M.F., Dias, J.M., 2015. Influence of synthetic antioxidants on the oxidation stability of biodiesel produced from acid raw Jatropha curcas oil. Fuel Process. Technol. 132, 133–138.

Szybist, J.P., Song, J., Alam, M., Boehman, A.L., 2007. Biodiesel combustion, emissions and emission control. Fuel Process. Technol., Biofuels for Transportation 88, 679–691.

Tang, H., Wang, A., Salley, S.O., Ng, K.S., 2008. The effect of natural and synthetic antioxidants on the oxidative stability of biodiesel. J. Am. Oil Chem. Soc. 85, 373–382.

Urbaniak, S.D., Caldwell, C.D., Zheljazkov, V.D., Lada, R., Luan, L., 2008. effect of cultivar and applied nitrogen on the performance of Camelina sativa L. in the Maritime Provinces of Canada. Can. J. Plant Sci. Rev. Can. Phytotech. 88, 111–119.

Veljković, V.B., Avramović, J.M., Stamenković, O.S., 2012. Biodiesel production by ultrasound-assisted transesterification: State of the art and the perspectives. Renew. Sustain. Energy Rev. 16, 1193–1209.

Vollmann, J., Moritz, T., Kargl, C., Baumgartner, S., Wagentristl, H., 2007. Agronomic evaluation of camelina genotypes selected for seed quality characteristics. Ind. Crops Prod. 26, 270–277.

Wall, J., Gerpen, V.J., Thompson, J., 2011. Soap and Glycerin Removal from Biodiesel Using Waterless Processes. Trans. ASABE 54, 535–541.

Wan Ghazali, W.N.M., Mamat, R., Masjuki, H.H., Najafi, G., 2015. Effects of biodiesel from different feedstocks on engine performance and emissions: A review. Renew. Sustain. Energy Rev. 51, 585–602.

Wan Omar, W.N.N., Saidina Amin, N.A., 2011. Optimization of heterogeneous biodiesel production from waste cooking palm oil via response surface methodology. Biomass Bioenergy 35, 1329–1338.

Westbrook, S., LeCren, R., 2003. Chapter 5—Automotive Diesel and Non-Aviation Gas Turbine Fuels, in: Totten, G., Westbrook, S., Shah, R. (Eds.), Fuels and Lubricants Handbook: Technology, Properties, Performance, and Testing. ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, pp. 115–144.

Wu, X., Leung, D.Y.C., 2011. Optimization of biodiesel production from camelina oil using orthogonal experiment. Appl. Energy 88, 3615–3624.

Xue, B., Luo, J., Zhang, F., Fang, Z., 2014. Biodiesel production from soybean and Jatropha oils by magnetic CaFe2O4–Ca2Fe2O5-based catalyst. Energy 68, 584–591.

Xue, J., Grift, T.E., Hansen, A.C., 2011. Effect of biodiesel on engine performances and emissions. Renew. Sustain. Energy Rev. 15, 1098–1116.

Yang, J., Corscadden, K., He, S.Q., Caldwell, C.D., 2015. The Optimization of Alkali-Catalyzed Biodiesel Production from Camelina sativa Oil Using a Response Surface Methodology. J. Bioprocess. Biotech. 05.

Yang, J., Caldwell, C.D., Corscadden, K., He, S.Q., Li, J., 2016. An evaluation of biodiesel production from Camelina sativa grown in Nova Scotia. Ind. Crops Prod. 81, 162–168.

Zubr, J., 2003. Qualitative variation of Camelina sativa seed from different locations. Ind. Crops Prod. 17, 161–169.

Zubr, J., 1997. Oil-seed crop: Camelina sativa. Ind. Crops Prod. 6.

APPENDICES

Copyright Permission (A):

Wed 2016-06-22 12:04 PM

Jie Yang

Co-authors' permission for the paper "An evaluation of biodiesel production from Camelina sativa grown in Nova Scotia"

To Claude Caldwell; Kenneth Corscadden; Quan.He@Dal.Ca; 'jili.li@dal.ca'

Dear all,

I am preparing my MSc thesis for submission to the Faculty of Graduate Studies at Dalhousie University, Halifax, Nova Scotia, Canada. I am seeking your permission to include a manuscript version of the following paper as a chapter in the thesis:

Paper title: An evaluation of biodiesel production from Camelina sativa grown in Nova Scotia.

Authors: Jie Yang, Claude Caldwell, Kenneth Corscadden, Quan Sophia He, Jili Li.

Paper status: Published in Industrial Crops and Products 81 (2016) 162-168.

Canadian graduate theses are reproduced by the Library and Archives of Canada (formerly National Library of Canada) through a non-exclusive, world-wide license to reproduce, loan, distribute, or sell theses. I am also seeking your permission for the material described above to be reproduced and distributed by the LAC(NLC). Further details about the LAC(NLC) thesis program are available on the LAC(NLC) website (www.nlc-bnc.ca).

Full publication details and a copy of this permission letter will be included in the thesis.

Please respond this email with your agreement/exception, also select/write down the granted choice (a or b) shown as below (please select b):

Permission is granted for:

a) the inclusion of the material described above in your thesis.
b) for the material described above to be included in the copy of your thesis that is sent to the Library and Archives of Canada (formerly National Library of Canada) for reproduction and distribution.

Yours sincerely,

Jie Yang

Wed 2016-06-22 12:05 PM

Kenneth Corscadden

Re: Co-authors' permission for the paper "An evaluation of biodiesel production from Camelina sativa grown in Nova Scotia"

To Jie Yang

Cc Claude Caldwell; Sophia He; Jili Li

You have my permission
Kenny

Sent from my iPhone

Wed 2016-06-22 12:08 PM

Sophia He

RE: Co-authors' permission for the paper "An evaluation of biodiesel production from Camelina sativa grown in Nova Scotia"

To Kenneth Corscadden; Jie Yang

Cc Claude Caldwell; Jili Li

You have my permission , Jack

Wed 2016-06-22 12:56 PM

Jili Li

Re: Co-authors' permission for the paper "An evaluation of biodiesel production from Camelina sativa grown in Nova Scotia"

To Jie Yang; Claude Caldwell; Kenneth Corscadden; Sophia He; Jili Li

---

you have mine too. thanks.

ELSEVIER LICENSE
TERMS AND CONDITIONS

Jun 22, 2016

This Agreement between Jie Yang ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	3894300912802
License date	Jun 22, 2016
Licensed Content Publisher	Elsevier
Licensed Content Publication	Industrial Crops and Products
Licensed Content Title	An evaluation of biodiesel production from Camelina sativa grown in Nova Scotia
Licensed Content Author	Jie Yang,Claude Caldwell,Kenneth Corscadden,Quan Sophia He,Jili Li
Licensed Content Date	March 2016
Licensed Content Volume Number	81
Licensed Content Issue Number	n/a
Licensed Content Pages	7
Start Page	162
End Page	168
Type of Use	reuse in a thesis/dissertation
Portion	full article
Format	both print and electronic
Are you the author of this Elsevier article?	Yes
Will you be translating?	No
Order reference number	
Title of your thesis/dissertation	EVALUATING THE FEASIBILITY OF BIODIESEL PRODUCTION FROM CAMELINA SATIVA
Expected completion date	Jul 2016
Estimated size (number of pages)	120
Elsevier VAT number	GB 494 6272 12
Requestor Location	Jie Yang Apt 11 317 College Rd Truro, NS B2N 2P6 Canada Attn: Jie Yang
Total	0.00 USD
Terms and Conditions	

INTRODUCTION

Copyright Permission (B):

Wed 2016-06-22 11:57 AM

Jie Yang

Co-authors' permission for the paper "The optimization of alkali-catalyzed biodiesel production from Camelina sativa oil using response surface methodology"

To Kenneth Corscadden; Quan.He@Dal.Ca; Claude Caldwell

Dear all,

I am preparing my MSc thesis for submission to the Faculty of Graduate Studies at Dalhousie University, Halifax, Nova Scotia, Canada. I am seeking your permission to include a manuscript version of the following paper as a chapter in the thesis:

Paper title: The optimization of alkali-catalyzed biodiesel production from *Camelina sativa* oil using response surface methodology.

Authors: Jie Yang, Kenneth Corscadden, Quan Sophia He, Claude Caldwell

Paper status: Published in the Journal of Bioprocessing & Biotechniques 2015, 5:7

Canadian graduate theses are reproduced by the Library and Archives of Canada (formerly National Library of Canada) through a non-exclusive, world-wide license to reproduce, loan, distribute, or sell theses. I am also seeking your permission for the material described above to be reproduced and distributed by the LAC(NLC). Further details about the LAC(NLC) thesis program are available on the LAC(NLC) website (www.nlc-bnc.ca).

Full publication details and a copy of this permission letter will be included in the thesis.

Please respond this email with your agreement/exception, also select/write down the granted choice (a or b) shown as below (please select b):

Permission is granted for:

a) the inclusion of the material described above in your thesis.
b) for the material described above to be included in the copy of your thesis that is sent to the Library and Archives of Canada (formerly National Library of Canada) for reproduction and distribution.

Yours sincerely,

Jie Yang

Wed 2016-06-22 11:57 AM

Sophia He

RE: Co-authors' permission for the paper "The optimization of alkali-catalyzed biodiesel production from Camelina sativa oil using response surface methodology"

To Jie Yang; Kenneth Corscadden; Claude Caldwell

Yes, you have my permission.

Wed 2016-06-22 12:04 PM

Kenneth Corscadden

Re: Co-authors' permission for the paper "The optimization of alkali-catalyzed biodiesel production from Camelina sativa oil using response surface methodology"

To Jie Yang

Cc Sophia He; Claude Caldwell

You have my permission
Kenny

Sent from my iPhone

Thu 2016-06-23 12:20 AM

Claude Caldwell

Re: Co-authors' permission for the paper "The optimization of alkali-catalyzed biodiesel production from Camelina sativa oil using response surface methodology"

To Jie Yang; Kenneth Corscadden; Sophia He

If there are problems with how this message is displayed, click here to view it in a web browser.

Hi,
This seems appropriate to me.
Claude

Copyright Permission (C):

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Jun 22, 2016

This Agreement between Jie Yang ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	3894311163255
License date	Jun 22, 2016
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	European Journal of Lipid Science and Technology
Licensed Content Title	The prediction of biodiesel storage stability. Proposal for a quick test
Licensed Content Author	Paolo Bondioli,Ada Gasparoli,Laura della Bella,Silvia Tagliabue,Florence Lacoste,Lionel Lagardere
Licensed Content Date	Dec 13, 2004
Licensed Content Pages	9
Type of use	Dissertation/Thesis
Requestor type	University/Academic
Format	Print and electronic
Portion	Figure/table
Number of figures/tables	1
Original Wiley figure/table number(s)	Figure 1
Will you be translating?	No
Title of your thesis / dissertation	EVALUATING THE FEASIBILITY OF BIODIESEL PRODUCTION FROM CAMELINA SATIVA
Expected completion date	Jul 2016
Expected size (number of pages)	120
Requestor Location	Jie Yang Apt 11 317 College Rd Truro, NS B2N 2P6 Canada Attn: Jie Yang
Publisher Tax ID	EU826007151
Billing Type	Invoice
Billing Address	Jie Yang Apt 11 317 College Rd Truro, NS B2N 2P6 Canada Attn: Jie Yang
Total	0.00 USD