SYNTHESIS AND EVALUATION OF POLYOL BASED BIOLUBRICANTS FROM VEGETABLE OILS

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by

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ABSTRACT

Vegetable oil is over 95% triacylglyceride (TAG) making it a potential low-cost feedstock for biolubricant production. The objective of this project was to develop a polyol-based biolubricant from vegetable oils with excellent oxidative stability and low temperature flow properties.

In the first study, a strong positive correlation was observed between saturated fat content and melting point while the content of polyunsaturated fatty acids (PUFA) was negatively correlated with oxidative stability. *Brassica rapa* cultivars, with less than 3.5% saturated fat and less than 20% polyunsaturated fat, can be an excellent feedstock with improved cold fluidity and oxidative stability.

In the second study, *B. rapa* TAG molecules were modified to produce fatty acid methyl ester (FAME) and then the acyl moieties were linked to trimethylolpropane (TMP) a branched neopentyl polyol by a two-step base-catalyzed transesterification reaction. The addition of FAME to TMP was explored and optimized by altering reaction protocols and catalysts. An efficient conversion (100%) of FAME and TMP to TMP triesters (TE) was successfully achieved under the optimum condition of 1 wt% potassium carbonate as the catalyst, 130 ºC reaction temperature, 18 h reaction time and a mole ratio of FAME to TMP of 3.9.

In the third study, the oxidative stability index (OSI) of the original vegetable oil, FAME and product TMP esters were all measured. The highest stability was observed in vegetable oil while the processed products were less stable. It is likely that natural antioxidants removed during purification of FAME and TMP esters contributed to the superior OSI value of the vegetable oil. The low temperature flow behaviour of TMP based biolubricants was determined between 298 K and 238 K using T2 relaxation. The results showed that the singlet attributed to TMP protons broadened until it disappeared as temperature decreased. The results indicated that the log of the spin-spin relaxation time is linearly correlated with rising temperature.

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LIST OF ABBREVIATIONS

AOCS: American Oil Chemists' Society

AOM: Active oxygen method

ASTM: American Society for Testing and Materials

CP: Cloud point

DAG: Diglyceride

DE: Diesters

FA: Fatty acids

FAME: Fatty acid methyl esters

FFA: Free fatty acids

GC: Gas chromatography

^{1}H-NMR: Proton Nuclear Magnetic Resonance

LEAR: Low erucic acid rapeseed

MAG: Monoglyceride

ME: Methyl esters

MW: Molecular Weight

MP: Melting point

NPG: Neopentyl glycol

OSI: oxidative stability index

PE: Pentaerythritol

PP: Pour point

PPD: Pour point depressant

PUFA: Polyunsaturated fatty acids

RP-HPLC: Reverse phase high performance liquid chromatography

T2: Spin spin relaxation time

TAG: Triglyceride

TE: Triesters

TMP: Trimethylolpropane

VI: Viscosity index

VM: Viscosity modifier

CHAPTER 1
INTRODUCTION

Modern lubricants are generally composed of more than 80% base oil and a smaller amount of functional additives (Arbain & Salimon, 2009). As a result, the base oil mainly determines lubricant properties such as oxidative stability, low-temperature flow properties and lubricity. There are three categories in base oils: mineral oils, synthetic oils and vegetable oils. Traditionally, over 85% of base oils are refined from crude petroleum (Yunus, et al., 2005); however, with the decreasing stocks of petroleum and high costs of synthetic lubricant, vegetable oils are considered to be potential candidates to supply high quality lubricant base oil for lubricant production. Vegetable oils have several advantages over other raw materials, as they are readily available, relatively low cost, renewable and environmentally friendly.

Although vegetable oils possess many desirable characteristics, currently they are not widely used as lubricant base oils. Largely this is due to undesirable physical properties of most vegetable oils, which include both a high melting point and insufficient thermal oxidative stability (Fox & Stachowiak, 2007). Natural triglycerides (TAGs), the major component of vegetable oils, consist of a glycerol backbone with three esterified long chain fatty acids. The physical properties of vegetable oils are strongly influenced by the glycerol structure and the fatty acid profile. On one hand, glycerol is not desirable in lubricants as the β hydrogen in glycerol triesters (TE) is unstable and susceptible to elimination reactions, which lead to degradation of the molecule. On the other hand, the geometry of TAG includes three ester groups arranged such that steric hindrance between the fatty acid groups is minimized. This arrangement may lead to organized packing of the molecules and formation of crystals during cooling. The instability and high melting point of vegetable oils maybe significantly improved by converting natural fatty acyl esters into synthetic esters (Itsikson, et al., 1969; Erhan, et al., 2006; Arbain & Salimon, 2009; Fox & Stachowiak, 2007; Campanella, et al.,2010). It has been reported that synthetic ester lubricating oils are widely applied in the production of lubricants for aircraft engines, because they can be used over a wide temperature range (Metro, et al., 1966). When vegetable oil is utilized as the

source of fatty acids for production of synthetic esters, a more resistant polyol may be chosen to replace the glycerol backbone. This replacement reaction requires at least two reactions, one to remove the glycerol and the second to esterify the polyol. Typically, branched polyols with neopentyl structure are more favoured for such syntheses because of the absence of β hydrogen in neopentyl polyol molecules. Neopentyl glycol (NPG) and trimethylolpropane (TMP) are two common polyols that have been used to produce synthetic esters lubricating oils.

Transesterification reaction may be categorized by the catalysts used. For example, acid catalysts, base catalysts and enzymes have all been used for transesterification. Enzyme catalyzed transesterification is a comparatively high-cost reaction and it is not commonly used in industrial production. Acid catalyzed transesterification is a one-step process that can produce polyol esters from polyol and free fatty acids. However, acid catalyzed reactions require a longer reaction time and may produce a lower yield of polyol esters than possible using a base catalyst (Itsikson, et al., 1967; Arbain & Salimon, 2009).

Base catalyzed production of polyol fatty acid esters typically requires two stages of transesterification when the initial feedstock is vegetable oil. From 1998 to 2011, there have been several reports of polyol esters synthesis using different TAG sources *via* two-step base catalyzed transesterification (Gryglewicz, et al., 2003; Rashid & Anwar, 2008; Yunus, et al., 2003; Yunus, et al., 2005). Methanolysis of TAG with alkali catalyst is a preferred first reaction that achieves a high reaction rate. This reaction proceeds to near completion due to the low solubility of glycerol in the methyl ester and effective removal of this production from the reaction. The second transesterification of fatty acid methyl esters (FAME) with NPG or TMP is, comparatively, a slower reaction. Driving this reaction near to completion requires high vacuum to remove the by-product methanol and excess of FAME to shift the equilibrium to the formation of polyol esters.

Both sodium and calcium methoxide are commonly used base catalysts for laboratory transesterification reactions due to their strong base properties. But these catalysts are not always the most suitable for industrial reactions. Methoxide catalysts are dangerous to handle on a large-scale due to their caustic properties, volatility and toxicity. Moreover, when compared with other common base catalysts such as potassium carbonate, potassium hydroxide and sodium carbonate, the price of reagent grade sodium methoxide is high. Therefore, potassium carbonate and potassium hydroxide can be more suitable base catalysts for industrial transesterification

reactions when high conversion efficiency can be achieved.

For the past twenty years, Proton Nuclear Magnetic Resonance (^{1}H-NMR) has played an important role in studying the structure of reactants and reaction products as well as monitoring oil chemical reactions. ^{1}H-NMR methods do not require chromatographic separations and can offer a rapid analysis of samples. In 1995, ^{1}H-NMR was first applied to study the kinetics and yield of transesterification reactions of rapeseed oil with methanol (Gerbard, et al., 1995). In FAME production, the transesterification process involves stepwise reactions to produce FAME from TAG molecules. Initially, TAG reacts with methanol to produce diacylglyceride (DAG) and FAME, then DAG reacts with methanol to produce monoacylglyceride (MAG) and FAME, and finally MAG reacts with methanol to release glycerol and FAME. The protons in TAG, DAG, MAG, methanol and glycerol may be monitored simultaneously due to their chemical shifts in ^{1}H-NMR. Conversion of TAG to FAME is readily tracked by ^{1}H-NMR as the appearance of the methoxyl group is observed as a singlet at 3.7 ppm. Consequently, integration of the signal arising from the methoxyl group protons is ideal for determining the production of FAME and monitoring reaction yield (Gerbard, et al., 1995).

In previous studies, ^{1}H-NMR was also used to confirm the production of TMP esters from FAME and TMP. The chemical shift arising from CH_2 protons of TMP is 3.45 ppm. However, when the hydroxyl group is esterified with a fatty acid, the electrons surrounding the protons in the CH_2 group are decreased. The lower electron density around the CH_2 group requires a weaker magnetic field to reach resonance conditions. Therefore, the signal arising from protons in the CH_2 group of TMP shifts to 4.02 and 4.00 ppm once TMP diesters (DE) and TE are formed.

The current study explores the correlation between fatty acid composition and oil melting point as well as oxidative stability, so as to identify the best fatty acid profile for lubricant base oil with both improved low-temperature fluidity and oxidative stability. After that, this study further develops and optimizes the production of polyol (TMP) esters from renewable vegetable oil by applying potassium carbonate catalyzed two-step base transesterification reaction. A lower cost and safer catalyst as well as improved reactions have been introduced in this study, so as to make the production more suited for industrial production.

In this study, the major objectives are as follow:

1. To determine the effect of fatty acid profile on biodiesel pour point (PP) and oil OSI of different oilseed species in order to establish the relationship between fatty acid composition and physical properties
2. To develop and optimize a two-step base-catalyzed transesterification reaction to chemically replace the glycerol backbone of plant TAG with a branched polyol called TMP using potassium carbonate as a safe and inexpensive catalyst
3. Synthesize TMP fatty acid esters from different vegetable oils *via* two-step base-catalyzed transesterification
4. To characterize the chemical structure of product and the production composition as well as monitoring the reaction conversion by applying ^{1}H-NMR
5. To characterize the polyol based biolubricants physical properties such as oxidative stability and low temperature flow properties
6. To apply a new method (^{1}H-NMR) to study the cold flow property of polyol based biolubricants at low temperatures

CHAPTER 2
LITERATURE REVIEW

2.1 The purpose of lubricant

A lubricant is a substance introduced between two surfaces in relative motion in order to reduce the friction between them and the wear induced by contact of the surfaces. Generally, many lubricants are solutions or colloids that include a lubricating solvent or base fluid with functional additives that improve its lubrication properties. Animal oils, vegetable oils, synthetic esters and mineral oils are the examples of modern lubricant base fluids.

Lubricants reduce both friction and wear between moving surfaces. The measure of the ability of a lubricant to reduce friction and wear is known as lubricity. By reducing wear, a lubricant extends the operational life of a surface and by reducing friction the energy required to move surfaces is reduced. In addition, lubricants transfer heat generated by moving parts, prevent corrosion and minimize the exposure of surfaces to corrosive compounds and wear particles (Haycock & Hillier, 2004). An excellent lubricant base fluid is required to have low volatility, excellent heat and oxidative stability, good cold fluidity and high viscosity index (Kuratomi & Nagano, 2009).

2.2 Historic development of lubricants

Mankind has used lubricants from the early days of civilization to assist in reducing the energy needed to slide one object against another (Lansdown, 2004). It is recorded that grease, oil, or mud have been utilized as lubricant as early as 2400 B.C. and liquid lubricant was valuable as the original lubricant for transporting sledges in the Sumerian and Egyptian civilizations (Persson, 2000). From the first century A.D., animal fats and vegetable oils were the principal lubricants used in machinery such as lathes, pulleys, gears (Doson, 1979). During the period of industrial revolution around 1760, heavy industrial iron and steel machinery, was widely introduced. Animal oils such as sperm whale oil and vegetable oil from sources such as palm and groundnut oil saw increased use as lubricants. At the same time, mineral oil obtained from the distillation of

coal, was developed for use as a lubricant. In the 1850s, small quantities of petroleum oil were produced in United States, Canada, Russia and Romania. Petroleum production, from oil wells in the United States, increased substantially by the 1880s (Haycock & Hillier, 2004). Subsequently, mineral oils accounted the greatest proportion of lubricant base oils. Erhan et al., (2006) reported that, 85–90% of the total global lubricant production originates from non-renewable petroleum precursors. However, with decreasing petroleum reserves and growing environmental pollution, conventional petroleum resources are no longer preferred for many lubricant applications. A trend has developed where fractionated and refined petroleum base fluid used in lubricant production is increasingly being replaced by synthetic stocks or modified plant base stocks.

2.2.1 Refined petroleum base oils

Petroleum is a natural product of decaying living organisms including plants, bacteria and animals (Tissot & Welte, 1978). Crude petroleum is a mixture that contains a large array of hydrocarbon molecules (such as paraffin, aromatics, cycloalkanes and alkenes) and small amounts of nitrogen, sulphur, oxygen, metal, and salt. Petroleum hydrocarbon molecules include covalently linked carbon atoms in an array of molecules with different carbon skeletons. Petroleum molecule carbon skeletons vary by chain length, branching patterns, cyclic structures and hydrogen saturation. The potential utility of hydrocarbons is, in part, determined by the molecular structure. Crude petroleum oil is refined by a number of processes that reduce the average molecular weight and remove atoms such as sulphur and nitrogen. Molecular weight reduction (cracking) and hydrogenation optimize the properties of petroleum. After cracking, groups of molecules are separated by fractional distillation, the separation of molecules based on boiling point. The lowest boiling fraction derived from petroleum comprises small alkanes with one to four carbon atoms. These compounds are readily separated from crude oil. They can be used for heating, cooking and for synthetic precursors for plastic. Lubricating oils are typically derived from fractions that boil at temperatures between 300 and 370 °C and typically include between 20 and 40 carbon atoms in each molecule (Haycock & Hillier, 2004). Molecules present in these fractions include long chain alkanes, cycloalkanes and aromatic compounds.

2.2.2 Synthetic base oils

Generally, synthetic oil is a mixture of compounds that are manufactured by chemical modification of petroleum. Synthetic oils are typically more stable to heat and oxidants than many mineral oils. In addition, synthetic oils are available with superior viscosity index and biodegradability when compared with mineral oils. As a result, certain synthetic fluids have become popular in the lubricant market. From 1985 to 2002, the synthetic lubricant proportion of total lubricant market for Western Europe rose gradually from less than 2% to over 10% (Haycock & Hillier, 2004).

Synthetic fluids are categorized by their composition or their chemical structure. Typical synthetic fluids include synthetic hydrocarbons (such as polymers of olefins, chlorinated hydrocarbons, condensation products and chemically modified mineral oils), polyether oils, esters, phosphoric acid esters, and oils containing silicon. Synthetic fluids have many advantages when compared with crude oils; however, the production cost for synthetic oil is generally four to eight times higher than mineral oil. The higher cost limits the market for synthetic oils (Rudnick, 2006). Nevertheless, synthesizing polyol esters of vegetable oil fatty acids might lead to a lower cost synthetic fluid (Yunus, et al., 2003; Arbain & Salimon, 2009; Uosukainen, et al., 1998). Useful vegetable oil materials are readily available and the price of vegetable oil would be reduced by the development of oilseeds that produce oil with enhanced stability in the future (Honary & Richter, 2011).

2.2.3 Vegetable oils

Vegetable oil can be categorized as edible and non-edible. Edible oil, such as canola, soybean and sunflower oil, are used both for human consumption but also for production of biodiesel and biolubricant. Non-edible oil, including waste cooking oil and seed oil from *Jatropha curcas* is also suitable for biofuel and biolubricant production (Patil & Deng, 2009). Historically, lubricants have been produced from both edible and non-edible vegetable oils.

Most vegetable oils are predominantly TAGs (Figure 2.1). TAG is a TE of glycerol where three fatty acids are linked to the three hydroxyl groups of glycerol *via* ester bonds. The physical and chemical properties of vegetable oil are largely contributed by fatty acid composition and distribution, in addition to the glycerol backbone structure in the TAG molecule (Rousseau, 2004). The film strength of a lubricant is a measure of its interaction with wear surfaces and is an

important measure of lubricant performance. TAG structure provides excellent lubrication because long fatty acid chains combined with polar carbonyl groups produce high strength lubricant films. Most vegetable oil TAGs contain fatty acids that range from 14 to 22 carbons with various degrees of saturation (Fox & Stachowiak, 2007). TAG molecules interact more strongly with the metallic surfaces than hydrocarbons, thus, they are often more effective in reducing both friction and wear as this property allows them to form a strong film on metal surfaces.

Vegetable oils may potentially substitute for mineral base oils in lubricant products. Vegetable oils are inexpensive and readily available, potentially allowing for production of lubricants that are less expensive than synthetic oil but more expensive than petroleum base oil (Rudnick, 2006). Some lower quality vegetable oils cannot be used for human consumption maybe discarded if not applied in non-food/feed products. Moreover, lower quality canola oil, which cannot be easily processed for human consumption may be sold for biofuel or biolubricant production. Increased markets for lower quality seed and oil could increase farmer incomes and maximize the application of agriculture products. In Canada, Agriculture and Agri-Food Canada has developed programs to support investment in biofuel production. The two programs (eco Agriculture Biofuels Capital Initiative and the Biofuels Opportunities for Producers Initiative) provide $200 million and $18 million respectively to support farmer participation in biofuel production (Honary & Richter, 2011).

Although they are not yet widely used as lubricant base oils, vegetable oils have many desirable characteristics for use as biolubricant base fluids. Vegetable oils tend to solidify at higher temperatures than desirable for many lubricant applications and may not be suited for direct use as lubricants. The low temperature flow properties of vegetable oil are influenced by the structure of the glycerol backbone as well as the fatty acid composition (Yunus, et al., 2005). The geometry of TAG includes three ester groups arranged such that steric hindrance between the fatty acid groups is minimized. This arrangement may lead to organized packing of the molecules and crystal formation during cooling. Furthermore, a high concentration of saturated fatty acids is not desirable as there is a strong correlation between saturated fatty acid concentration and melting point. Oil that contains predominantly mono- or polyunsaturated fatty acids have a lower melting point than those that are rich in saturates. For example, the PP of palm oil based synthetic lubricant increased from –30 to –9 ºC when the amount of palmitic acid (C16:0) acid rose above 10%, w/w (Yunus, et al., 2005).

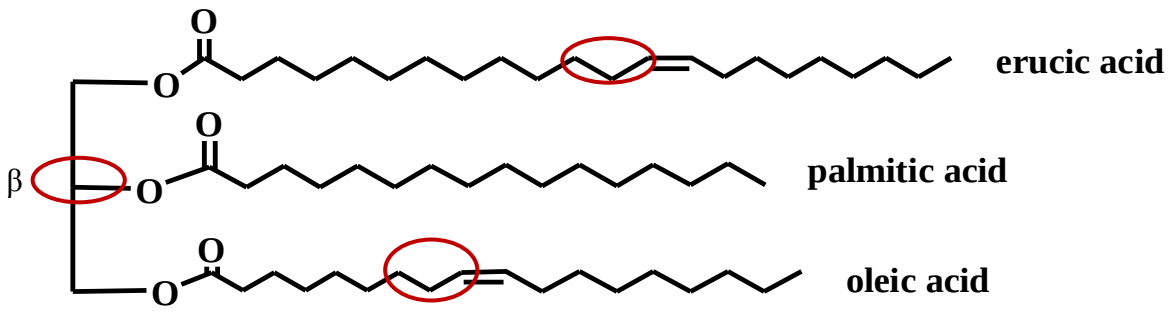
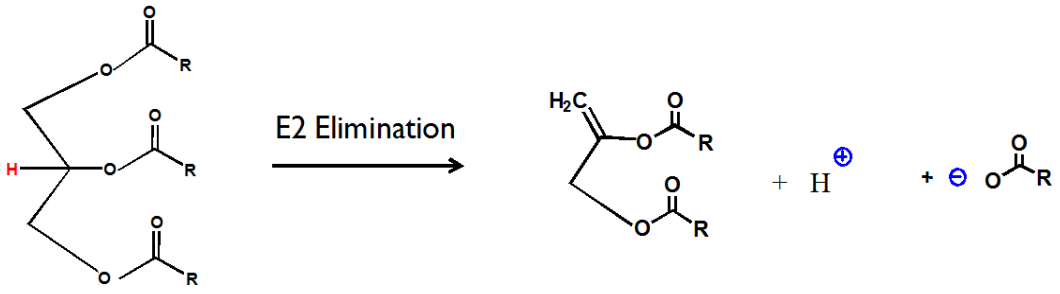


Figure 2.1 Chemical structure of a TAG [a] molecule and oxidation susceptible sites

[a] triglyceride

In addition to poor low temperature performance when compared with petroleum and synthetic base oils, vegetable oil has comparatively poor oxidative stability. Both the structure of glycerol and the fatty acid composition of TAG oils contribute to low oxidative stability. As shown in Equation 2.1, the hydrogen atoms on the β carbon of the glycerol backbone are susceptible to elimination reactions that would lead to degradation of the molecule.



[2.1]

In addition, vegetable oils may be oxidized when they are heated or exposed to the air. The number of PUFA affects the oxidative stability of the vegetable oil. Therefore, vegetable oil stability is inversely proportional to the relative unsaturation of the fatty acids. Canola and rapeseed oils contain intermediate percentages of the PUFA [linoleic (C18:2) and linolenic (C18:3) acids]. The interrupted diene structure of double bonds of these fatty acids is able to participate in oxidation reactions and their presence largely determines oil oxidative stability (Fox & Stachowiak, 2007). To summarize, high saturation of lipids leads to superior oxidative stability, but poor low flow temperature flow properties, while the presence of PUFA improves low temperature performance, but decreases oxidative stability.

Researchers have taken two approaches to improve the properties of vegetable oil for use as lubricant base oil with improved flow property and oxidative stability. As shown in Figure 2.2, the fatty acids are removed from the glycerol and attached to another more stable polyol such as NPG, TMP and pentaerythritol (PE) (Bongardt, et al., 2000; Rudnick, 2006; Honary & Richter, 2011). In another method epoxidation has been used to improve the oxidative stability in rapeseed oil based lubricant (Wu, et al., 1998). As well, genetic modification and selective hydrogenation were also considered in a previous study (Hery & Battersby, 1998). In addition, oils with the ideal fatty acid profile for both low temperature performance and oxidative stability may be selected. The relationship between oil fatty acid profile, low temperature flow properties and oxidative stability has been intensively investigated. It was noted that vegetable oils with a high percentage of

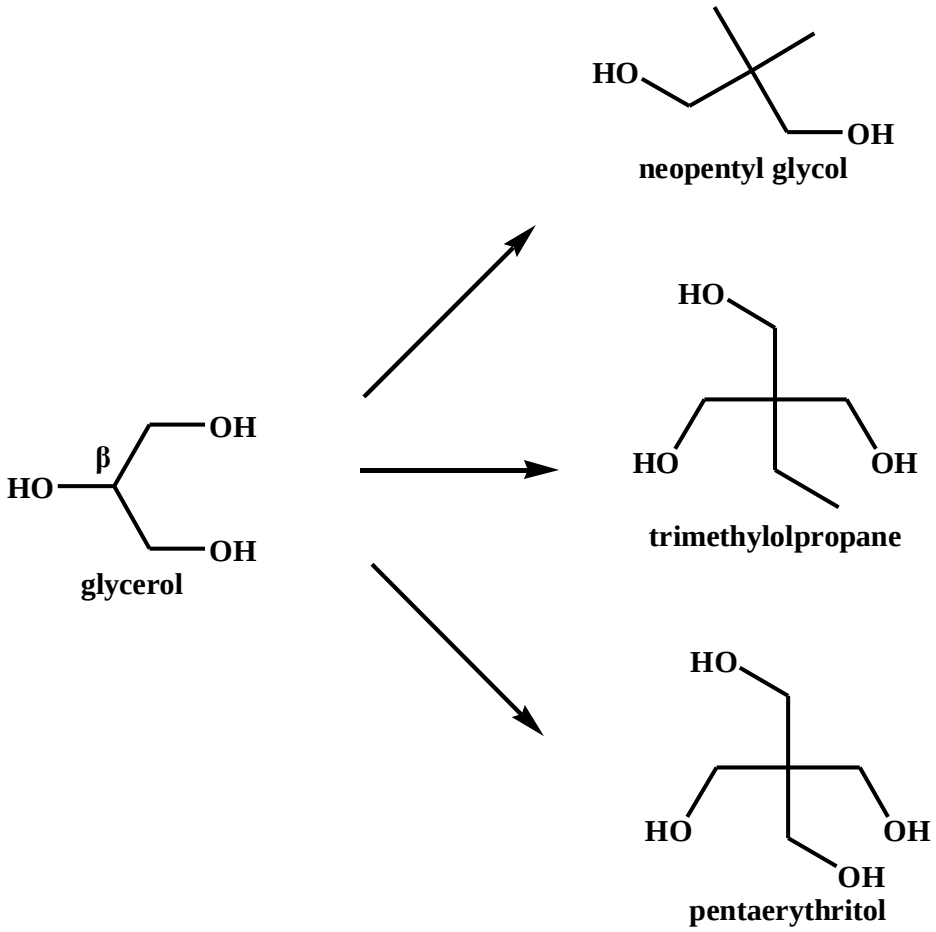
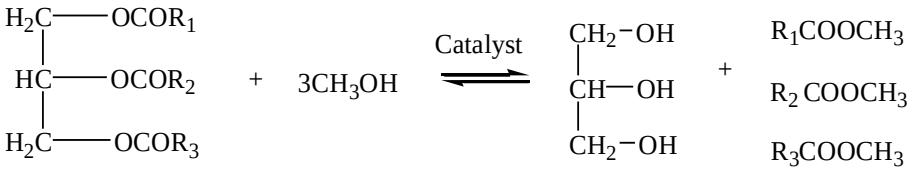


Figure 2.2 Branched polyols with neopentyl structure

mono-unsaturated fatty acids are the most likely candidates to achieve both improved low temperature performance and oxidative stability (Bongardt et al., 2000; Fox & Stachowiak, 2007). It is worth mentioning that vegetable oils such as rapeseed oil, sunflower oil and soybean oil all have substantial concentrations of oleic acid, an eighteen carbon mono-unsaturated fatty acid.

2.3 Fatty acid methyl esters of vegetable oils

Fatty acid methyl esters (FAME) are produced by transesterification of TAG oil (usually from animal fats or vegetable oils) with methanol in the presence of catalyst as described in Eq. [2.2].



Triglyceride Methanol Glycerol Methyl esters [2.2]

FAME can be used as fuel for diesel engines in either its pure form or blended in all portions with petroleum diesel. When used to fuel diesel engines FAME is also called biodiesel. Kim, et al., (2004) reported that for every kilogram of biodiesel used to replace diesel fuel 3.2 kg of carbon dioxide emissions were avoided. Therefore, biodiesel as a biodegradable, non-toxic and renewable fuel shows the potential to be an alternative of petroleum. While it is desirable to select the ideal fatty acid composition for biodiesel production, oil derived from inexpensive and available feedstock is preferred for biodiesel production. Furthermore, the cost of oil for FAME feedstock varies among countries. For examples, soybean oil is commonly chosen for FAME production in North America, while waste-cooking oil is utilized in China.

2.3.1 Production of FAME

Transesterification is commonly used to produce FAME, in which esters are susceptible to reaction with alcohols in the presence of a catalyst so that the alcohols are exchanged. Transesterification of TAG is a stepwise and reversible process in which DAG and MAG are produced as intermediates in the reaction of alcohol with TAG (Meher, et al., 2006). When

methanol is used in synthesis of biodiesel, TAG molecules react with methanol to produce DAG and methyl ester. In a subsequent reaction, DAG reacts with a second molecule of methanol to form MAG and a second molecule of methyl ester. In a final reaction, MAG reacts with a third molecule of methanol to yield one mole of methyl ester and glycerol. The following Eq. [2.3] shows this consecutive and reversible process:

$$\begin{aligned} &\text{Triglyceride} + R_1OH \rightleftharpoons \text{Diglyceride} + ROOR_1 \\ &\text{Diglyceride} + R_1OH \rightleftharpoons \text{Monoglyceride} + ROOR_1 \\ &\text{Monoglyceride} + R_1OH \rightleftharpoons \text{Glycerol} + ROOR_1 \end{aligned} \qquad [2.3]$$

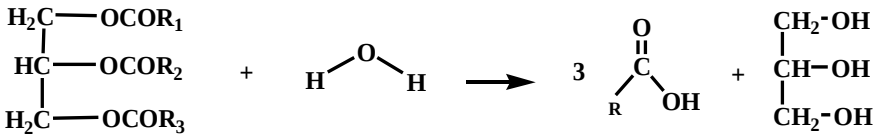
The stepwise reactions are reversible and an excess of alcohol is used to shift the equilibrium towards the formation of esters. In the presence of excess alcohol, the forward reaction is pseudo-first order and the reverse reaction is found to be second order. As well, it was concluded that important factors such as reaction temperature, reaction type, amount of catalyst, the ratio of alcohol to oil, the mixing intensity and reaction time could affect the transesterification reaction rate (Rashid & Anwar, 2008).

2.3.2 Variables of transesterification reactions

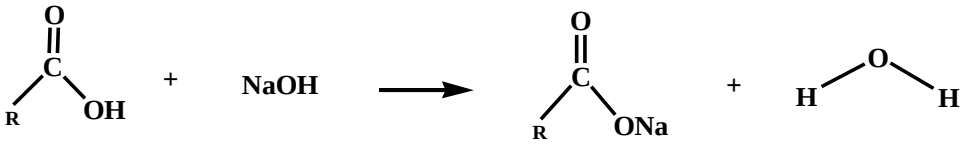
Alkali, acid and enzyme catalysts can catalyze the transesterification reaction. The reaction occurs at a faster rate when catalyzed by alkali catalysts such as sodium methoxide, potassium hydroxide and sodium hydroxide (Leung, et al., 2010; Helwani, et al., 2009; Lam, et al., 2010).

In base catalyzed transesterification, the free fatty acid (FFA) and moisture content are important parameters in reactions as they can result in side-reactions such as hydrolysis as shown in Eq. [2.4] and saponification as shown in Eq. [2.5]. Moisture can react with TAG to release the FFAs and glycerol, and then FFAs can react with alkali catalyst to form soap. It is recommended that the FFA value should be less than 3% in order to enable an effective base catalyzed reaction. Increased acidity of the oil decreases the efficiency of conversion of TAG to esters (Meher, et al., 2006). Wang, et al. (2009) recommended the use of acid catalyzed esterification as illustrated in Eq. [2.6] to esterify FFA to FAME so as to lower acidity (from 66.40 mg KOH/g to 3.36 mg KOH/g) of waste cooking oil that is high in FFA prior to base catalyzed transesterification

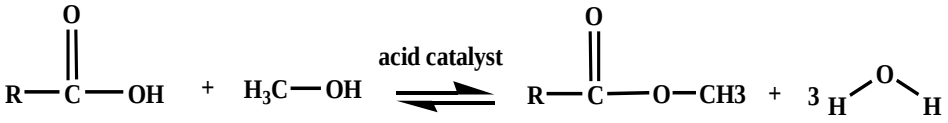
reactions.



TAG water FFA Glycerol [2.4]



FFA sodium hydroxide soap water [2.5]



Free fatty acids methanol FAME water [2.6]

In previous studies, both excess and insufficient base catalyst can significantly influence the reaction. Reactions of vegetable oil with 1% of either sodium hydroxide or potassium hydroxide catalyst gave the best yield of ester (Meher, et al., 2006). Rashid and Anwar (2008) investigated the effect of potassium hydroxide concentration varying between 0.25 and 1.50% on transesterification. Using 1% catalyst or more produced the highest methyl ester yield (96%). Nevertheless, the conversion is not increased with further increases in base catalyst concentration. The maximum yield of canola oil transesterification with methanol is achieved by 1 wt% addition of potassium hydroxide while yield of ester decreased with increasing catalyst concentration. It was proposed that excess base catalyst causes soap formation, which significantly influences the reaction (Patil & Deng, 2009). Furthermore, separating excess catalyst from the reaction products raises the production cost of biodiesel (Leung & Guo, 2006).

The molar ratio of alcohol to oil is one of the key factors affecting the yield of biodiesel. Theoretically, three moles of alcohol and one mole of TAG react to yield three moles of FAME. Since this reaction is stepwise and reversible, excess of alcohol is necessary to shift the equilibrium towards the formation of esters. Meher et al. (2006) concluded that a molar ratio of 6:1 should be used to achieve maximum conversion to FAME. When the molar ratio of methanol to oil was raised from 3:1 to 6:1, the yield of biodiesel dramatically increased from 57 to 96% (Rashid & Anwar, 2008). However addition of higher molar ratios such as 9:1 and 12:1 required longer equilibration time to separate methyl ester from glycerol.

Other factors such as reaction temperature, reaction time and mixing intensity are also important to achieve the optimum conditions for FAME production from TAG. In previous studies, 93 to 98% conversion rate was achieved after 1 h reaction time at 60 ºC reaction temperature (Meher, et al., 2006; Leung & Guo, 2006). As well, increased mixing intensity might be used to increase the contact area between oil and catalyst methanol solution. Conversely, it was observed that 300 rpm and 600 rpm gave the same yield of methyl esters in the reaction (Rashid & Anwar, 2008).

2.3.3 Purification of methyl esters

The transesterification reaction products are a mixture containing FAME, catalyst, excess methanol and by-product glycerol. When transesterification is complete, the reaction mixture separates into two phases. A low-density low polarity phase consisting of FAME and methanol maybe separated from a higher density polar phase that contains the glycerol, excess methanol and catalyst. In industrial facilities methanol is recycled by distillation of both phases after the reaction. Catalyst and methanol remaining in the FAME phase may be removed by rinsing with water (Rashid & Anwar, 2008; Uosukainen, et al., 1998). The crude FAME may also be refined by molecular distillation (Model MD 80), in which a solution of colorless FAME is produced (Wang, et al., 2010).

2.4 Fatty acid polyol esters of vegetable oils

Fatty acid polyol esters of vegetable oils are a potential bio-lubricant that may be derived from renewable sources. Modified vegetable oil esters are environmentally friendly and rapidly biodegradable base stock for lubricant production (Wagner, et al., 2001). Polyol esters are

produced by transesterification of vegetable oil fatty acids or fatty acid esters and polyol such as TMP, NPG and PE. The polyols (shown in Figure 2.2) are all branched polyols that have no labile hydrogen atoms in their structure that are equivalent to the β carbon of glycerol. Therefore, esters of these polyols have greater thermally stability than glycerol esters of the same fatty acids.

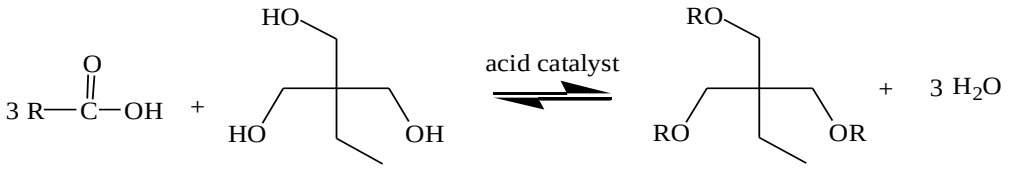
TMP is a colourless triol (three hydroxyl groups) with the formula $CH_3CH_2C(CH_2OH)_3$. This compound is often used to produce TE compounds that can substitute for TAGs in lubricants (Arbain & Salimon, 2009; Schneider, 2006) and produce vegetable oil based lubricants with improved properties. Previously, Yunus et al. (2005) synthesized TMP esters with palm oil fatty acids and fractions of palm oil (*Elaeis sp.*) fatty acids. The product materials were reported to have improved PP (as low as –37 ºC). In a separate study, Arbain and Salimon (2009) reported that the PP of *Jatropha curcas* TAG oil was 10 ºC, while the TMP TE of *Jatropha curcas* fatty acids had a PP of –37 ºC.

2.4.1 Production of polyol esters

Several synthetic approaches are available for the transesterification reaction between FAME and polyol, which produces polyol ester. Such reactions have been classified by the catalysts used in the reactions. Acid, base or enzyme catalysts are used for esterification and transesterification reactions (Schuchardt, et al., 1998). The optimization of enzyme-catalyzed transesterification has been investigated in many studies; however, it is not commercially available (McNeill, et al., 1991; Zaks & Gross, 1990).

2.4.1.1 Acid catalyzed esterification

Acid catalyzed esterification of fatty acids with TMP is shown in Eq. [2.7].



[2.7]

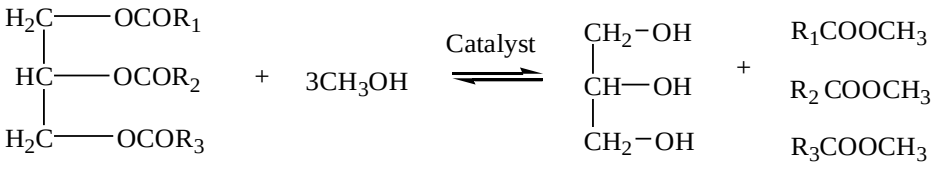
Itsikson et al. (1967) studied acid-catalyzed esterification of TMP with isovaleric and n-valeric acids. They reported that 85% of the free alcohol groups were esterified when 7% sodium

bisulphate was used as the catalyst at 110–120 ºC for 2 h while 83% was obtained using 1% sulphuric acid as the catalyst for a 60 h reaction time. Recently, Arbain and Salimon (2009) utilized a one-step sulphuric acid catalyzed esterification to produce TMP esters of *Jatropha curcas* oil. Under the reaction conditions (4:1 mole ratio of fatty acids to TMP, 2% sulphuric acid as the catalyst, temperature of 150 ºC for 3 h) partial conversion (55%) of the reactants to TMP esters was observed. The major problem encountered in the acid-catalyzed esterification is slow reactions that lead to long reaction time and low yield of TMP TE.

2.4.1.2 Base catalyzed transesterification

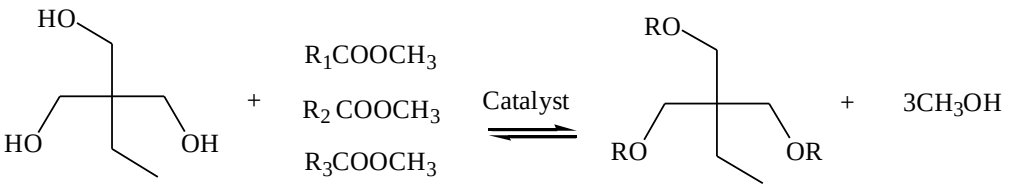
Base catalyzed production of polyol fatty acid esters from TAG typically requires two stages of transesterification. The first transesterification, as described in Eq. [2.8], is the process of using an alcohol (commonly methanol or ethanol) in the presence of a catalyst to chemically cleave TAG molecules of the raw vegetable oil to fatty acid esters. The co-product of this first reaction is glycerol. The second stage reaction for production of polyol fatty acid esters as shown in Eq. [2.9] with a chemically resistant polyhydric alcohol (such as isosorbitol or neopentyl polyols, including PE, TMP, or NPG) yields a product suitable for testing as a lubricant.

Stage One:



Triglyceride Methanol Glycerol Methyl esters [2.8]

Stage Two:



Trimethylolpropane Methyl esters Trimethylolpropane ester Methanol [2.9]

Common alkaline catalysts such as sodium hydroxide, potassium hydroxide, sodium methoxide, sodium carbonate and potassium carbonate are have all been used for base catalyzed transesterification. Sodium methoxide has been studied in the second stage of base catalyzed transesterification and was reported as the most effective catalysts in many studies (Uosukainen et al., 1998; Yunus et al., 2003; Gryglewicz et al., 2003). However, sodium methoxide may not be the most suitable catalyst for industrial scale reactions as it is easily inactivated. Sodium methoxide powder can react with air rapidly when added to the reaction system. Moreover, it is expensive, caustic, volatile and toxic. For instance, the price of reagent grade sodium methoxide (99% purity) is $2,300 US per ton, while sodium hydroxide (99% purity) is 400 US per ton and potassium hydroxide (92% purity) is $770 US per ton (Leung & Guo, 2006). Therefore, the costs associated with base catalyzed transesterification with sodium methoxide may be reduced by developing reactions with safer and lower cost base catalysts such as potassium hydroxide or potassium carbonate.

High conversion efficiency of FAME to polyol ester is possible. For example, it has been reported that 99% of TMP was successfully converted to TE in the transesterification reaction of low erucic acid rapeseed (LEAR) oil methyl ester with TMP (Uosukainen, et al., 1998). In this reaction 0.5% sodium methoxide was used to catalyze the reaction of 3.3 mole ratio of LEAR oil methyl ester to TMP at 110–120 ºC for 10 h under 3.3 pKa pressures. Subsequently, Yunus et al. (2003) optimized the reaction time and produced 98% TMP TE with 0.9% sodium methoxide as the base catalyst at 130 ºC for 1 h at a pressure of 20 mbar and 3.9 mole ratio of palm kernel oil methyl esters to TMP.

An improved two-step transesterification process for producing higher polyol (sucrose) fatty acids polyesters (Volpenhein, 1985) was developed. In the first step of the reaction high conversion to polyol esters (90%) was achieved by using potassium carbonate as the base catalyst, while 85% conversion was obtained by using sodium methoxide. The ratio of FAME to sucrose in the first reaction was 4.75 and in the second reaction stage was 7.98. Therefore, this base catalyzed transesterification was efficiently catalyzed using a safer and less expensive base catalyst (potassium carbonate) to achieve high percentage of conversion.

2.4.2 Purification of polyol esters

The production of polyol esters of fatty acids from methyl esters and polyol produces a crude product mixture that includes unreacted FAME, fully esterified polyols, smaller amounts of partially esterified polyols and catalyst. Commonly, these crude products could be washed with water and acid or alkaline solutions to remove the catalyst, and then further refined by distillation to remove the unreacted methyl esters (Arbain & Saliman, 2009; Yunus, et al., 2003). Heating the reaction mixture in an oil bath (180–200 ºC) under reduced pressure (0.2 mbar) enabled the removal of unreacted esters from the product. However, there are some disadvantages in using high temperature distillation to purify the crude product; for example, PUFA might undergo conjugation during high temperature distillation.

2.4.2.1 Reversed phase C-18 high performance liquid chromatography

Reversed phase C-18 high performance liquid chromatography (RP-HPLC) is a common analytical method used for separating crude mixed materials. It has been reported that TAG, FA and FAME have all been separated and quantified by RP-HPLC (Aveldano, et al., 1983; Plattner, et al., 1976). Therefore, it might be a possible to separate the TMP TE and excess FAME in the reaction product mixture using RP-HPLC.

Reversed phase chromatography is a process based on the partitioning interaction between solute molecules in a chromatographic mobile phase and a stationary phase. In a reversed phase column, the stationary phase (the immobilised non-polar ligand) may be any of a broad range of non-polar groups bonded to a particle in the column. For example, silica beads with porous surfaces are widely used as a support for column chromatography. The silica surfaces of the beads are often modified by octadecyl carbon chains (C18). In such chromatographic columns, the distribution of the solute between the two phases depends on the binding properties of the medium, the polarity of the solute and the composition of the mobile phase. Analytes with lower polarity are better retained in the non-polar phase. Therefore, a less polar analyte will spend more time within the non-polar phase than a more polar analyte.

Reversed phase chromatographic separation efficiency depends on the reversible adsorption and desorption of the solute molecules with different polarity to a non-polar stationary phase. The column (packed with the reversed phase medium) is equilibrated under suitable initial mobile phase conditions of pH, ionic strength and polarity. The polarity of the mobile phase is

adjusted by precise control of the ratio of two or more solvents of different polarity such as acetone and acetonitrile. The mobile phase must be able to dissolve the partially non-polar solute to allow interaction of the solute with the reversed phase chromatographic matrix. The sample is typically dissolved in the mobile phase that is used to equilibrate the column in the first step. The dissolved sample is applied to the column through an injector and carried onto the column with the mobile phase that is adjusted at a flow rate where optimum binding will occur. Once the sample is introduced to the chromatographic bed it is typically washed with further amounts of the mobile phase used to equilibrate the column. This helps to remove unretained solute molecules. The retained solutes are then desorbed from the reversed phase medium by adjusting the polarity of the mobile phase so that the bound solute molecules will sequentially elute from the column. Note that substances that are not desorbed during the analysis must be removed. Near complete removal of all bound substances is achieved by washing with a low polarity solvent. The column may then be re-equilibrated with the solvent or combination of solvents initially used in readying the column for injection of additional samples.

It can be hypothesized that preparative reversed phase chromatography of crude reaction product mixtures containing TMP TE, DE, as well as the FAME can yield a fraction that is highly enriched in TMP TE based on its low polarity compared to other compounds in the fraction. Moreover, reversed phase HPLC on octadecylsilyl column has been successfully applied to separate FAME and FA using acetonitrile-water mixtures as a mobile phase (Aveldano, et al., 1983).

2.5 Quantification of FAME and biolubricant yield

2.5.1 Chromatographic methods

Design of reactions for the efficient synthesis of any product requires the ability to monitor reaction products. There are many potential methods that may be applied to the analysis of reactions of methyl esters with polyol. Chromatography has been used a quantification method in many previous studies. Both Yunus et al. (2003) and Uosukainen et al. (1998) determined reaction progress by monitoring the levels of fatty acids methyl esters, fatty acids and TMP as well as TMP ME, DE and TE by thin layer chromatography and gas chromatography or high performance liquid chromatography.

2.5.2 Nuclear Magnetic Resonance spectroscopy quantification

For the past twenty years, Proton Nuclear Magnetic Resonance (^{1}H-NMR) has played an important role in studying the structure of reactants as well as monitoring chemical reactions. Spectral methods that do not require chromatographic separations and can offer rapid sample analysis. In addition, the sample may not require any purification steps in advance of the analysis. ^{1}H-NMR was first applied to study the yield of transesterification of rapeseed oil with methanol (Gerbard, et al., 1995). Later, ^{1}H-NMR method was also applied to monitor transesterification reactions of soybean oil (Morgenstern, et al., 2006). In FAME production, the transesterification process involves stepwise reactions to produce FAME from TAG molecules to DAG and FAME, then conversion of DAG to MAG and FAME, and a final conversion of MAG to glycerol and FAME. As shown in Figs. 2.3 and 2.4, the protons in fatty acids display different chemical shifts in ^{1}H-NMR. Once the fatty acids are converted to FAME, they produce a distinctive methoxyl group proton signal at 3.7 ppm. Consequently, the signals of methoxy groups in the methyl esters at 3.7 ppm (singlet) were ideal for quantifying the transesterification yield (Gerbard, et al., 1995). Similarly, the proton signal arising from the α-methylene groups present in all fatty ester derivatives at 2.3 ppm (triplet) can be used to determine the total amount of fatty acid and ester present throughout a reaction. As illustrated as following equation, the yield for the transesterification reaction is obtained directly from the areas of the selected signals using Eq. [2.10].

$$Y\% = 100 \times (2A1/3A2) \qquad [2.10]$$

Where A1 = areas of the methoxy group (3.7 ppm, singlet); and A2 = area of the α-methylene group (2.3 ppm, triplet). Values are calculated at ± 2% according to the reproducibility of integration.

In previous studies, ^{1}H-NMR was used to analyze the structure for TMP esters as shown in Figure 2.5. The chemical shift of protons in the TMP-CH_2 group is 3.45 ppm. However, when the hydroxyl group is attached to a fatty acid, the electron density around the protons in the -CH_2 group was decreased. Therefore, the protons in that CH_2 group shifts to 4.02 ppm and 4.00 ppm once TMP DE and TE are formed.

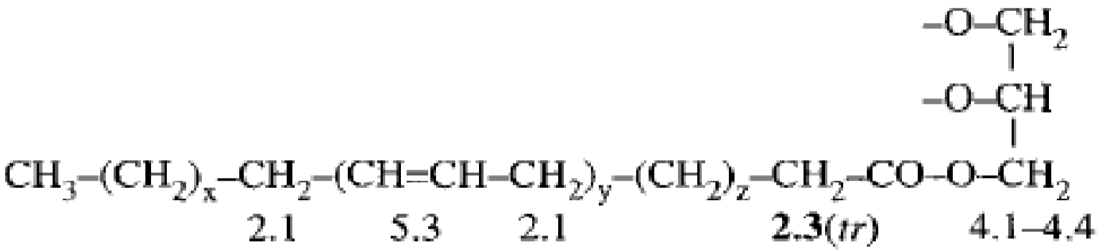


Figure 2.3 Chemical shifts of TAG[a] protons
[a] triglyceride

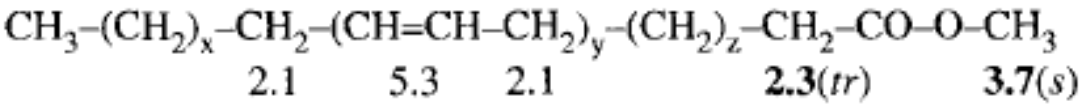


Figure 2.4 Chemical shifts of FAME [a] protons
[a] fatty acid methyl ester

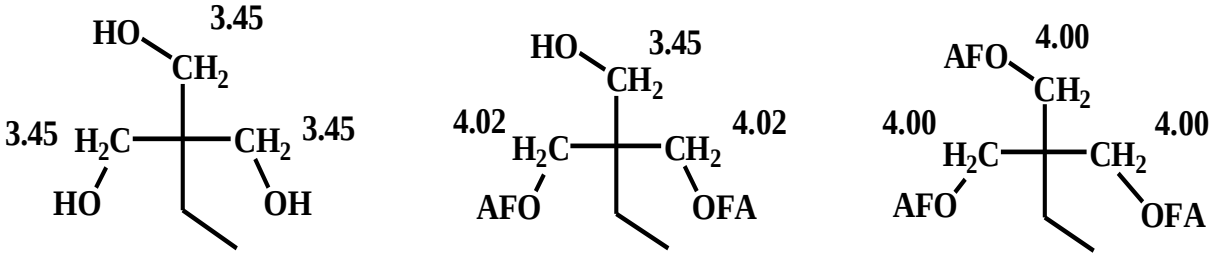


Figure 2.5 Chemical shifts of protons for TMP [a], TMP DE [b] and TMP TE [c]
[a] Trimethylolpropane, [b] Trimethylolpropane diesters, [c] Trimethylolpropane triesters

2.6 Characterization of esters and functional additives

Many properties of lubricants are common physical properties like viscosity, stability and low temperature flow properties. These properties are generally expressed by quantitative parameters such as viscosity index, OSI, PP and cloud point (CP). Each parameter represents one of the lubricant characteristics and a standard method is available to measure the characteristic. These physical properties of lubricants are mainly determined by the oil structure and chemical composition. Typically the base molecules lack the ideal properties for use as lubricants hence modern lubricant base oils are normally blended with functional additives to improve their physical properties.

Oil additives are materials (usually chemical compounds) that are added in small amounts to lubricant base oils to improve their properties. In the 1930s, additives were developed and applied to improve the petroleum based oil performance (Haycock & Hillier, 2004). At that time, additives were only used to improve the physical properties of the lubricants, such as to increase oxidative stability and decrease engine deposits. However, conventional petroleum base stocks have become less able to satisfy the increasingly stringent lubricant requirements. Therefore, modern lubricants are now highly specialized and complex mixtures to which many functional additives are used to modify a wide range of properties. The main families of additives are buffers, antioxidants, detergents, anti-wear compounds, corrosion inhibitors, friction modifiers, viscosity index improvers and PP depressants.

2.6.1 Viscosity and viscosity modifiers

Viscosity, as one of important properties of lubricating oils, can be termed as the property of resistance to flow in a fluid or semi-fluid (Diaz, et al., 1996). Viscosity index (VI) is generally used to characterize viscosity. There are two measures of the VI of lubricating oils called kinematic viscosity and dynamic viscosity. A capillary tube viscometer may be used to measure the kinematic viscosity of transparent and opaque liquids (ASTM D445). This method determines the time required for a specific volume of liquid to flow under gravity through a calibrated glass capillary viscometer. It is typically reported at two temperatures, 40 ºC (100 ºF) or 100 ºC (212 ºF) in the units of centistokes (cSt), equivalent to mm^2/s in SI units. Viscosity decreases with increasing temperature, decreasing molecular weight, acyl chain length and branching. The viscosity of crambe oil is 51.5 cSt at 40 ºC and 10.5 cSt at 100 ºC. TMP esters produced from

crambe oil have high viscosity (56.9 cSt at 40 ºC and 11.0 cSt at 100 ºC; Rudnick, 2006).

Viscosity modifiers (VMs) are oil-soluble polymer molecules that decrease in volume when cold and increase in volume when hot. In doing so, they maintain the viscosity of the oil as it heats up, whereas a fluid without VMs would typically lose viscosity more quickly. VMs increase the VI as they are more soluble in the base stock as high temperature than at low temperature. Usually, the concentration of polymer in high-VI base oils may be from 0.5 to 2% (Haycock & Hillier, 2004).

2.6.2 Low temperature fluidity and PP depressants

Low temperature fluidity is an important physicochemical property of lubricating oils, especially where cold winter temperatures can drop to –40 to –50 ºC. PP is the lowest temperature at which oil can flow or pour when tilted in a test jar. It is a good indicator to predict the low temperature performance of an oil product and its operability in cold weather conditions. The PP is strongly influenced by oil structure and fatty acid ester composition. For example, PP decreases with increasing number of double bonds in the molecule; therefore, unsaturation is desirable for cold flow properties. Furthermore, branching is another factor that affects the PP of oils. In previous studies, chemical modification was suggested to reduce crystallization in vegetable oils (Rudnick, 2006). For example, the PP of *Crambe* oil decreases from –9 to –12 ºC and further to –27 ºC when the glycerol backbone of the *Crambe* oil was replaced by TMP and isopropyl alcohol, respectively (Rudnick, 2006).

Cloud point (CP) is another indicator of oil cold flow behaviour and it is the temperature that the high melting components of the oil start to crystallize making the oil turbid. When oil is cooled crystals may agglomerate and grow in size until the oil solidifies. A fuel or lubricant that is above its PP but below its CP may plug filters and lines (Haycock & Hillier, 2004). Both PP and CP are also strongly influenced by the oil source and structure.

When chemical modification is not sufficient to produce a lubricant with suitable low temperature flow properties PP depressants (PPDs) are commonly used lower the PP. For instance, it was reported that the lowest temperature of vegetable oil flow can be decreased from –15 to –30 ºC using suitable PPDs (Rudnick, 2006). PP depressants are polymeric molecules that are applied to lubricants to improve their low-temperature flow properties and modify wax crystals in the oils (Haycock & Hillier, 2004). For example, paraffinic base stocks are derived from crude petroleum

and contain molecules that have linear carbon chains of 14 carbons or more. These waxy species crystallized in large plates that link together to form a network that prevents oil flow. The poor flow properties decrease the ability of the engine oil circulation system to pump the oil at low temperatures. PPD can control the wax crystallization phenomenon by both delaying the formation of wax-gel matrix that would normally occur and reducing the viscosity contribution of the crystal wax particles. Most PPDs act by interrupting the three-dimensional growth of wax crystals (Haycock & Hillier, 2004). Paraflow, the first branded synthetic PPD additive, was commercialized in 1932.

2.6.3 Oxidative stability and anti-oxidants

When oil is exposed to air or high temperature it is prone to oxidize. Factors such as heat, light, metal catalysts, water, and acids influence this process. During oxidation the oil becomes darker and thicker. Severely oxidized oil will form deposits. Oxidation produces undesirable compounds, which eventually compromise the lubricant properties; these compounds shorten service life and, under extreme circumstances, damage the machinery requiring lubrication. As shown in Figure 2.6, free radical chain reactions are initiated by oxygen after which the radicals undergo additional reactions including propagation, branching and termination (Dong & Migdal, 2009).

In Figure 2.6 the compound undergoing reaction is given as RH. Where the reactant is TAG an unsaturated fatty acyl group would be RH. In an initiation reaction, removing a hydrogen atom on the fatty acid molecule forms a free radical R•. This process is generally slow but can be accelerated by heat, light and metal ions such as copper, iron and manganese. Subsequently the free radical R• may react with oxygen to form a peroxy radical ROO•. This reaction is rapid and the peroxy radical ROO• propagates to attack the hydrogen from another RH to produce another free alkyl radical R• and hydroperoxide ROOH. This process, unless terminated, will occur each time a free radical R• is produced. In a chain branching process, hydroperoxide ROOH would be decomposed into an alkoxy radical RO• and a hydroxyl radical OH•. These radicals could participate in further reactions to form volatile compounds such as alcohol, aldehydes, ketones and hydrocarbons. This reaction may form alkyl radicals that accelerate the oxidation and generate further aldehydes and ketones, significantly altering the lubricating oil. In typical reactions viscosity decreases while volatile products increase. In the end, the reaction terminates when two

radicals react to form a covalently linked non-radical compound.

Oil oxidative stability can be measured *via* two main kinds of test: predictive tests and oxidation indicator tests (Velasco & Dobarganes, 2002). These tests are used to predict the effect of minor compounds such antioxidants on oil stability.

Predictive tests are applied to determine the amount of oxidation that can occur under normal operating conditions. Common methods include the Schaal oven test, active oxygen method (AOM) and OSI (Velasco & Dobarganes, 2002). The Schaal oven test determines the development of rancidity of oil in a heated (63 ºC) sealed glass tube. AOM is based on AOCS method 12−57. In this method the oils heated in a glass tube and samples are taken periodically to determine the peroxide value. When the PV reaches 100 meq/kg the length of time is recorded as a measure of oxidative stability.

Commercially available equipment is used in determining the OSI as described in AOCS method Cd 12b-92. OSI is defined as the point of maximum change of the rate of oxidation, which is believed to occur when most antioxidants are lost from the oil. According the American Oil Chemists' Society method, OSI may be determined by recording the level of volatile acidic oxidation products over time. During typical oxidation, the accumulation of oxidation products is slow initially followed by a sudden increase in accumulation of these products. The OSI is determined by trapping the volatile acids from oxidizing oil in distilled water and repeatedly measuring the conductivity of the water.

OSI is usually presented in units of time (h) before the rate of oxidation begins to accelerate. The longer the time before accelerated accumulation of oxidation products is observed the more stable the sample. Typically, OSI analysis is conducted at elevated temperatures (100 to 130 ºC) and under constant airflow (a stream of purified air is passed through a sample of oil). Degradation of the TAG molecule leads to the formation of volatile organic acids. Air that has been passed through the oil sample contains the organic acids. This air is passed through deionised water where accumulation of organic acids increases the conductivity of the water. Conductivity electrodes connected to a computer are used to monitor changes in conductivity. The measurement is complete after a rapid increase in the rate of accumulation of organic acids is observed. This is called the induction point. The time period between the start of analysis and the induction point is the OSI.

Anti-oxidants can inhibit oil oxidation and terminate the oxidation reaction. Natural oils contain some anti-oxidants that can inhibit oxidation; however, modern refining can remove many natural anti-oxidant compounds. It has been reported that high quality petroleum base stocks were readily oxidized because heavy refining removes certain phenolic and amine products have the ability to inhibit oil oxidation (Haycock & Hillier, 2004). TAG oils are also susceptible to oxidation and the rate of oxidation is affected by the degree of fatty acid unsaturation and presence of natural or added antioxidants. As oxidation may be slow in the presence of antioxidants they are important additives to base oils. Many effective antioxidants have been developed and used. The major classes of antioxidant additives are compounds containing sulphur, phosphorus, aromatic amines, and hindered phenolic compounds (Dong & Migdal, 2009).

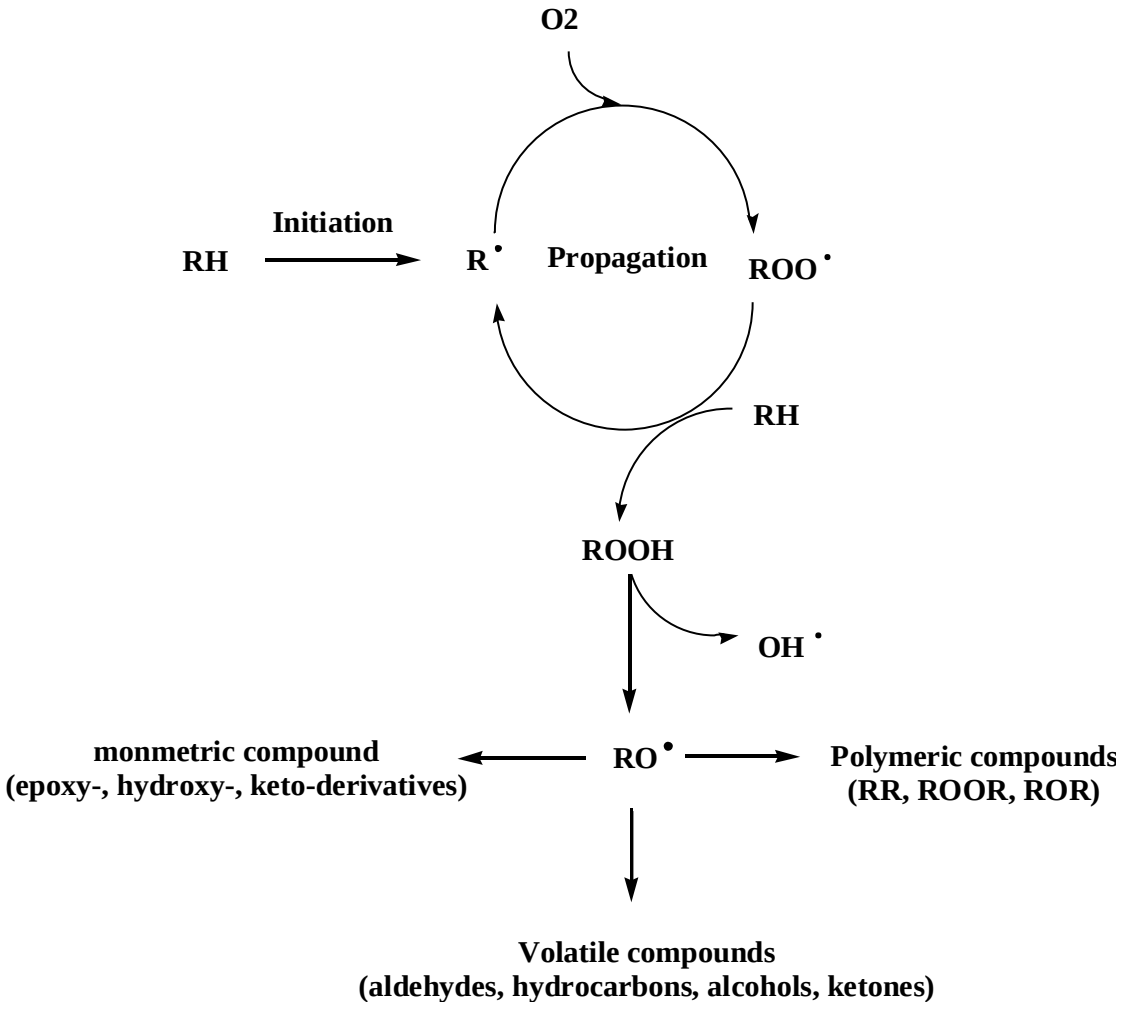


Figure 2.6 Process of auto-oxidation (Modified from Velasco and Dobarganes, 2002)

CHAPTER 3
EFFECT OF FATTY ACID PROFILE ON OIL PHYSICAL PROPERTIES

3.1 Abstract

Many oilseeds are being produced in Canada but it is not known which of these produces oil with the lowest temperature flow properties and the greatest oxidative stability. In this study oil samples were obtained from five cultivars of *B .rapa* L., seven cultivars of *Brassica napus* L. and five cultivars from other oilseeds species namely as *Brassica. juncea* L., *Sinapis alba* L., *Camelina sativa* L. and *Linum usitatissimum* L. The fatty acid profile, CP and PP were determined for each oil sample. Fatty acid methyl esters (FAME) were synthesized from each oil sample. For oil samples obtained from *Brassica* sp. the saturated fatty acid content was highly correlated (93.4%) with PP. The content of saturated fatty acids [combined palmitic (C16:0) and stearic (C18:0) acids] in *B. rapa* cultivars is lower (varying from 2.59 to 4.04%) than that observed in other vegetable oils. Since *B. rapa* oil exhibited superior low temperature properties, it was chosen for further studies of oxidative stability. Seeds of over 2,000 individual plants of *B. rapa* cv. Echo were harvested and the fatty acid profile was determined by gas chromatography (GC). Subsequently, seeds with similar content of PUFA were pooled to generate larger samples of 20, 21, 22, 23 and 24% combined linoleic (C18:2) and linolenic (C18:3) acids. Oil was extracted from the pooled samples using n-hexane as the solvent. A negative correlation was found between the level of PUFA and OSI in the pooled samples. A decrease in combined C18:2and C18:3 (from 24 to 20%) was associated with a doubling in the oil oxidative stability (OSI from 6 to 12 h). A combination of low saturated fat and low polyunsaturated fat content are key factors providing oil with excellent low flow property and oxidative stability. *B. rapa* cultivars, with less than 3.5% saturated fat and less than 20% polyunsaturated fat, can be an excellent feedstock for production of oils with improved cold fluidity and oxidative stability.

3.2 Introduction

Vegetable oil is a renewable and environmentally friendly alternative for producing fluid products normally made from fossil hydrocarbons like fuel, dielectric fluids and lubricants. Most vegetable oils are primarily TAG in which three fatty acids are linked to three hydroxyl groups of glycerol *via* ester bonds. However, the use of unmodified vegetable oil in lubricants and biofuels is often limited due to undesirable properties including poor cold flow performance and oxidative stability. These physical and chemical properties are largely contributed by fatty acids in the TAG that vary in saturation and chain length (Rousseau, 2004).

CP and PP are two important indicators to predict the fluidity and operability of oil in cold weather conditions. The temperature at which crystals first appear in a fluid hydrocarbon product is called the CP and the temperature at which a sample of the oil will not flow is called the PP. If the temperature drops below the CP crystals that form at the CP can plug filters and lines and, thereby, block flow. As the temperature decreases further crystals agglomerate and grow in size until the hydrocarbon product does not flow, as it is no longer a fluid. Generally, FAME made from vegetable oils, which are often used as a fuel, have a CP between –6 and 13 ºC and a PP ranging from –15 to 0 ºC (Srivastava, et al., 2000; Imahara, et al., 2006; Ramadhas, et al., 2005; Dmytryshyn, et al., 2004; Dunn, 2009). The high degree of unsaturation contributes a lower melting point (Imahara, et al., 2006) and long carbon chain saturated methyl esters (ME) provide higher PP (Ramo, et al., 2009). Saturated fatty acids with chain length higher than C12 substantially increase the PP (Knothe, 2005; Yunus, et al.,2004). Many studies showed that decreasing saturated fat content is associated with improved oil PP at lower temperatures. Dunn (2011) and Gryglewicz et al. (2003) demonstrated that fractionation improved ME by reducing the high-melting saturated fatty acids. In addition, compounds present at low concentrations may influence low temperature behaviour. Sterol glycosides reduce fluidity at low temperature in biodiesel made from soybean oil, since it has high melting point (MP) (240 ºC) and low solubility in biodiesel or diesel fuel (Lee, et al., 2007). The concentration of sterol glycosides is lower in corn, sunflower and canola oil than soybean oil (Lee, et al., 2007). Monoglycerides (MAGs) have also been found in biodiesel. Low temperature filtration of FAME has recovered small amounts of MAGs of saturated fatty acids. The presence of saturated MAGs in ME products is proportional to the amount of saturated fat in the TAG. Clearly determining the presence of minor compounds is critical to understand the low temperature performance of a TAG its ester products.

The variation in concentration of minor compounds among oils taken from different plants is large. Therefore, it is difficult to study the effect of fatty acid composition on low temperature flow and oxidative stability among the oil products obtained from different oilseed species.

The oxidation stability of TAGs and products derived from TAGs decreases with increasing unsaturation (Ramo, et al., 2009; Falk, et al., 2004; Imahara, et al., 2006). PUFAs contribute most to oxidative instability. In addition, non-TAG components such as tocopherols, carotenoids, free fatty acids and sterols are also key factors that influence oxidative stability (Neff, et al., 1994). It is necessary to remove non-TAG compounds before studying the effect of fatty acid composition on oil oxidative stability (Hawrysh, 1990).

Generally, the composition of fatty acids in vegetable oil plays an important role in determining both cold fluidity and stability of the oil and modified products derived from the oil. For example, a high content of long chain saturate fat such as palmitic (C16:0) and stearic (C18:0) acids contribute to oil good stability, but poor cold fluidity. In addition, PUFA such as linoleic (C18:2) and linolenic (C18:3) acids give to lower PP but poor stability. Improved physical and chemical properties have been achieved by increasing the content of monounsaturated fatty acids, particularly those made from oleic acid (Ramo, et al., 2009; O'Keefe, et al., 1993; Rudnick, et al., 2006). This study was designed to identify the best oil composition for achieving excellent cold fluidity and stability by investigating the impact of fatty acid composition on physical and chemical properties of oil derived from different oilseeds.

3.3 Materials and methods

3.3.1 Materials

Fivecultivars of *B. rapa* (Span, Torch, Arlo, Polish and Echo) and seven cultivars of *B. napus* (46A65, Midas, Oro, Weststar, Golden, Argentine, and Nugget) were obtained from Agriculture & Agri-Food Canada (Saskatoon, SK, Canada). *B. juncea*, *S. alba* and *C. sativa* were provided by Peacock Industries (Hague, SK, Canada).Two cultivars of *L. usitatissimum* were supplied by Crop Development Center, University of Saskatchewan (Saskatoon, SK, Canada).

Hexane (98.5% HPLC grade), NaOH (99%), BF_3 (14% in methanol) and KOH were supplied by EMD Chemicals (Gibbstown, NJ, USA). Methanol (99% HPLC grade), NaOMe (95%) and NaCl (99%) were obtained from Sigma-Aldrich (St. Louis, MO, USA), Internal

standard of C23:0 methyl ester was obtained from Nu-check Prep Inc. (MN, USA) while hydrogen gas was purchased from Praxair Canada Inc. (Ontario, Canada).

3.3.2 Solvent oil extraction

Five cultivars of *B. rapa* (Span, Arlo, Torch, Polish and Echo) and five cultivars of *B. napus* (Midas, Oro, Golden, Argentine and Nugget) were extracted using hexane for 5 h on a BUCHI Universal Extraction System B-811 (BUCHI Analytical Inc. New Castle, DE, USA). The oil was recovered by evaporating hexane followed by heating in an oven at 80 ºC.

Echo seed (70 g) was ground in a coffee grinder (model 843; Moulinex, Scarborugh, ON, Canada). The ground Echo seed was transferred to a 1 L beaker and slurried in hexane (400 mL). The slurry was stirred using a Teflon-coated bar at 800 rpm overnight. Subsequently, the light yellow slurry was filtered using a Büchner funnel. The hexane in the filtrate was removed using a rotary evaporator (model Rotavapor R-200; BUCHI, New Castle, DE, USA) at 50 ºC. The sample was held under vacuum (0.5 torr) at room temperature overnight to remove the hexane residues.

3.3.3 Mechanical oil extraction

Oil from two cultivars of *B. napa* (46A65, Weststar) and five other plant oilseeds (*B. juncea*, *S. alba*, *C. sativa* and two cultivars of *L. usitatissimum*) were mechanically extracted on a Komet Press (Model: CA 59G3, IBG Monforts, Oekotec GmbH & Co., KG, Germany)while applying heat through the electric cuff supplied with the press. The drive speed setting of 5 to 6 was used for all extractions. Crude oils obtained were filtered through a fiberglass filter disk (Whatman International Ltd., Maidstone, England) in a Buchner funnel to remove particulate matter.

3.3.4 Fatty acid profile analysis

Fatty acid profiles of oils from solvent extraction and pressing were determined according to AOCS Official Method Ce 1b-89. Gas chromatography (GC) analysis was performed using Agilent Technologies 6890 Network GC system. GC chromatogram was obtained using DB-WAX with a capillary length of 10m, a film thickness of 0.20 μm and a diameter of 0.100 mm. Hydrogen was applied as the carrier gas and 0.2 μL was injected by Agilent 7683 Series Injector.

Fatty acid profiles of seeds were analyzed by the following procedures: 100 mg seed was weighed in a 2 mL Eppendorf tube, then 1mL of internal standard C17:0 solution in hexanes was added. Samples were shaken for 1 min using a Retsch Shaker, then centrifuging for 5 m at 10,000 rpm using 5417C countertop centrifuge. The upper layer was transferred to another Eppendorf tube and 0.5 mL 3% sodium methoxide was added. Over the next 30 m the sample was mixed with a mechanical agitator and later 1.0 mL 20% citric acid in water solution was added. Then sample was centrifuged for 2 m at 10,000 rpm using 5417C countertop centrifuge. The upper layer was filtered through 0.45 μm PTEE filter into a GC vial for GC analysis by DB-WAX column see above.

3.3.5 Sterols contents in the oils

Sterols contents in the oils were estimated through the ^{1}H-NMR spectroscopy (Bruker AMX-500 spectrometer, Rheinstetten, Germany, operating at 500 MHz at 23 ºC, solvent: $CDCl_3$). Integration area of sterols in oil ^{1}H-NMR spectra refers to integration value of sterol C18 protons at approximately 0.68 ppm, after calibrating the integration value of the terminal -CH_3 protons to 3.00. Sterols contents in the oils (% w/w) were thus calculated from oil ^{1}H-NMR spectra based on the following formula:

$$W_o = \frac{3}{9} \times FW_o \quad [3.1]$$

Where W_o = relative oil weight and FW_o = average oil formula weight which is 880.

$$W_s = \frac{As}{3} \times FW_s \quad [3.2]$$

Where W_s = relative sterol weight, A_s = integration area of sterols in oil ^{1}H-NMR spectra, and FW_s = average sterol formula weight which is 405.

Therefore, the sterol content in oil was calculated from Eq. [3.3]

$$S = \frac{W_s}{W_o} \times 100 \quad [3.3]$$

Where S = sterols in oils (% w/w)

Sterol esters content in biodiesel samples was also estimated through ^{1}H-NMR spectroscopy using the following equations:

$$W_b = \frac{3}{3} \times FW_o \quad [3.4]$$

Where W_o = relative biodiesel weight and FW_o = average oil formula weight which is 290.

$$W_s = \frac{As}{3} \times FW_s \quad [3.5]$$

Where W_s = relative sterol weight, A_s = integration area of sterols in biodiesel oil ^{1}H-NMR spectra, and FW_s = average sterol ester formula weight which is 625.

$$S = \frac{W_s}{W_o} \times 100 \quad [3.6]$$

Where S = sterols esters in biodiesel (% w/w)

3.3.6 Fatty acid methyl ester synthesis

The filtered oils obtained from various oilseeds were converted to FAME using a two-stage alcoholic (methanol) transesterification process, followed by several purification steps. The oil was reacted with 12% by weight of a solution of potassium hydroxide in methanol (0.5% w/w) at 22 ºC for 1.5 h. The upper phase of the reaction mixture (FAME) was reacted for a second time with 8% by weight of the same solution for 1.5 additional hours. The upper phase of the second reaction was washed with water twice (3% w/w) and the resulting upper phase was dried in the presence of silica gel 60 (EMD Chemicals Inc., Germany). The FAME was filtered using a glass fiber filter to remove solids.

3.3.7 Cold flow properties

The CP and PP of purified FAME synthesized from various oilseeds were determined. FAME samples (25 mL) were placed into a glass test tube (26 mm diameter). The test tube was closed with a one-hole stopper that held a thermometer in the sample. The test tube containing the sample was then put into a 100 mL volumetric cylinder that was then placed into a refrigerated bath. There was no direct contact between the test tube and the cooling media. The experimental settings and measurement procedures largely followed those of standard method ASTM D97

(ASTM, 2011). Sample test tube was taken from the volumetric cylinder starting from 0 ºC every 3 ºC of cooling and was inspected visually for a haze which was the CP. The CP was determined as the temperature at which high MP substances began to crystallize producing a haze in the oil. The PP was determined by adding 3 ºC to the temperature at which the sample would not flow if tilted on its side for five seconds.

3.3.8 Oxidative stability

OSI was determined using an Oxidative Stability Instrument-24 (Omnion, Hingham, MA, USA). Deionized water (50 mL) was added to the polycarbonate conductivity tubes and conductivity probes were inserted into the water. Samples (5.0 ± 0.2g) were weighed and added directly to each glass reaction tube. The heating block was adjusted to 110 ºC. Glass tubes containing samples were inserted into the heating block and connected to the air manifold and to the polycarbonate tubes, containing deionized water. The pressure of the air manifold was 38,000 Pa and airflow was started at the beginning of the test. During oxidation, the purified air was continuously bubbled into the sample tube. Any volatiles produced by oxidation would flow into the conductivity tube. As a result, the conductivity of the deionized water would increase due to accumulation of volatile oxidation products indicating oxidation reaction. Changes in conductivity were measured over time.

3.4 Results and discussion

3.4.1 Oil fatty acid profile and ME properties

Fatty acid profiles of oil samples from *B .rapa, B. napus* and other oilseeds are presented in Table 3.1. There are three main types of fatty acids in the plant seed oils: saturated (typically combined C16:0 and C18:0), monounsaturated (typically C18:1, C20:1 and C22:1), polyunsaturated (typically combined C18:2 and C18:3). Different cultivars of *B. rapa* have similar compositions with characteristically low saturated fat content (1.88 to 2.93% C16:0 and 0.71 to 1.22 % C18:0) and medium polyunsaturated content (16.3 to 21.6% C18:2; 7.79 to 10.2% C18:3). Similar findings were observed in *B. napus* cultivars; however, the saturated fatty acids were relatively higher than those in *B. rapa* cultivars. Cultivars from *B. rapa* and *B. napus* are were both rapeseed and canola types. These cultivars have been developed with large differences

in their erucic acid (C22:1) content. Canola cultivars with below 1.03% C22:1 content, had higher levels of C18:1, while rapeseed cultivars with high C22:1 content had lower saturated fat content of C16:0 and C18:0.

CPs and PPs of FAMEs synthesized from extracted seed oil samples are summarized in the Table3.2. Generally, *B. rapa* cultivars had the lowest PP ranging from –21 to –18 ºC followed by *B. napus* cultivars varying from –18 to –12 ºC. Other vegetable oils including *B. juncea*, *S. alba*, *C. sativa*, and two cultivars of *L. usitatissimum* had the highest PP from –13 to –8 ºC. *B. rapa* cultivars had the potential for use in producing superior oils with excellent low-temperature flow properties. The cultivar Echo, a selection taken from Polish, which produced ME with a PP of –21 ºC, was chosen for a study of oxidative stability.

3.4.2 Saturated fat and ME PP

The range of saturated fatty acids in the current study was lower (2.59 to 9.32 %) than other previous studies (7 to 44.7 %) (Erasmus, 1993; Ramo et al., 2009). Also, ME derived from *B. rapa* and *B. napus* oils had lower PPs (–21 to –12 ºC) than those derived from other seed oils (–15 to 0 ºC) in previous studies (Srivastava, et al., 2000; Imahara, et al., 2006; Ramadhas, et al., 2005; Dmytryshyn, et al., 2004; Dunn, 2009). Linear regression analysis showed a strong linear relationship (Figure 3.1) between the saturated fatty acids contents (combined C16:0 and C18:0) and ME PP with correlation coefficient (R^2) of 0.93 in *B. rapa* and *B. napus* ME. Increasing the saturated fatty acid contents from 2.59 to 5.84 % resulted in marked increase of the PP of ME from –21 to –12 ºC. Especially, the ME of *B. rapa* cultivars (Arlo, Polish and Echo) oils had the lowest PP (–21ºC) and contained the least amount (≤ 3.5 %) of high-melting components (C16:0 and C18:0). These results generally agreed with previous studies (Knothe, 2005; Imahara, et al., 2006; Ramo, et al., 2009) with respect to the role of saturated fatty acids affecting ME PP. Pure MEs of C18:1, C18:2, C18:3 and C22:1 have MP below 0 ºC. Conversely, ME of C16:0 and C18:0 have MP of 28.5 ºC and 37.7 ºC. These saturated MEs are the first components that precipitate during cooling (Dunn, 2011; Mittelbach & Remschmidt, 2004). Therefore, a lower concentration of C16:0 and C18:0 is associated with a lower onset temperature of crystallization. From the strong linear relation between saturate fat content and ME PP in Figure 3.1, the ME PP could be even lower than –21 ºC, if a cultivar with less than 2.59% saturate fat is identified.

Table 3.1 Fatty acid profile (%) of oil in different oilseeds and cultivars

Seed	Cultivar	C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1	Others[a]	Total
B. rapa	Arlo	1.88	0.71	27.51	16.26	7.79	8.93	33.04	3.88	100
	Polish	2.04	0.87	35.5	17.46	8.03	9.19	23.41	3.5	100
	Echo	2.28	1.22	32.13	16.94	8.77	11.34	23.94	3.38	100
	Span	2.93	1.11	62.93	21.6	9.01	1.13	1.03	0.26	100
	Torch	2.48	1.1	64.47	19.97	10.22	1.05	0.58	0.13	100
B. napus	Golden	3.17	1.22	19.18	14.36	7.66	9.57	39.48	5.36	100
	Argentine	3	1	18.52	14.62	10.14	8.7	39.2	4.82	100
	Nugget	2.99	1.15	18.78	13.64	8.11	9.55	40.74	5.04	100
	West star	3.79	1.93	65.45	17.09	7.19	2.03	1.01	1.51	100
	46A65	3.72	2.03	67.3	18.1	6.65	1.21	0	0.99	100
	Midas	3.76	1.76	68.45	18.87	5.81	0.74	0.19	0.42	100
	Oro	4.03	1.81	67.77	19.87	5.24	0.84	0	0.44	100
B. juncea	Vulcan	3.18	1.5	23.98	23.86	11.36	11.39	19.48	5.25	100
S. alba	Andante	2.81	1.06	24.89	9.21	10.8	10.63	34.94	5.66	100
L. usitatissimum	N.A.	6.11	3.21	13.23	75.1	2.34	0	0	0.01	100
	N.A.	4.81	3.51	19.19	15.96	56.53	0	0	0	100
C. sativa	N.A.	4.9	2.18	12.76	16.71	37.47	15.1	3.15	7.73	100

[a]Values for other fatty acid contents were obtained by subtracting determined fatty acids from 100.

Table 3.2 Properties of methyl esters from all vegetable oil samples

Seed	Cultivar	CP(ºC)	PP(ºC)	Sterols esters* (%w/w)	
				Oil	FAME
B. rapa	Arlo	-2	-21	0.91	0.99
	Polish	-3	-21	0.94	1.46
	Echo	-3	-21	1.48	1.56
	Span	-7	-18	1.4	1.45
	Torch	-7	-18	1.26	1.41
B. napus	Golden	0	-18	1.08	1.39
	Argentine	-2	-18	1.29	0.99
	Nugget	-1	-18	0.95	1.02
	West star	-5	-13	1.62	1.04
	46A65	-5	-12	1.45	1.08
	Midas	-4	-12	1.28	1.14
	Oro	-4	-12	1.74	1.37
B. juncea	Vulcan	-4	-11	1.66	1.13
S. alba	Andante	-4	-13	1.46	1.16
L. usitatissimum	N.A.	-5	-10	0.58	0.61
	N.A.	-5	-8	0.44	0.73
C. sativa	N.A.	-2	-11	1.2	0.79

*Expressed as a mixed sterol ester with an average molecular weight of 625 Daltons

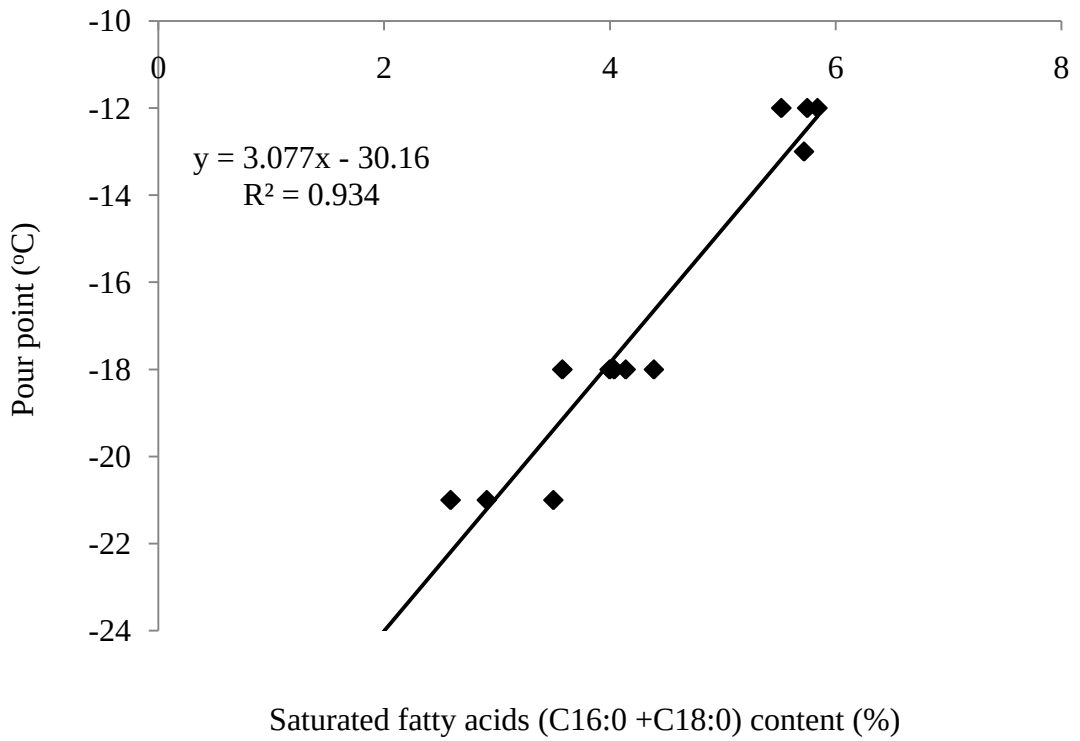


Figure 3.1 Relationship between combined saturated fatty acids and pour points of methyl esters produced from *B. rapa* and *B. napus* cultivars

3.4.3 Monounsaturated fat and ME PP

The presence of methyl erucate (C22:1) with a MP of –3.05 ºC might limit the low temperature flow properties of biodiesel (Knothe and Dunn, 2009). Both *B. rapa* and *B. napus* cultivars, oilseeds with medium C22:1 (23.41 to 33.04%) had the lowest ME PP at –21 ºC (Arlo, Polish and Echo), while cultivars having either low (<1.03%) or higher C22:1 (39.48 to 40.74%) had ME PP between –18 and –12 ºC (described in Figure 3.2). The very strong correlation observed between saturate content and PP suggests that erucic acid is not determining PP. The observed low PP of oils from varieties with medium C22:1 was likely due to the low saturate content of these oils as a result of oilseed metabolism. Furthermore, freezing point depression may occur between structurally similar lipids. C22:1 and C18:1 are both *cis*-monounsaturated fatty acids and structurally similar. During nucleation, C22:1 fatty acid molecules may crystallize. C18:1 FAME molecules are structurally similar to C22:1 FAME and thus may have affinity for the growing crystal. However, these two fatty acids are not identical; therefore, FAME from 18:1 can interfere with the crystallization of C22:1 FAME. This finding was consistent with Sari et al. (2004) and Sari (2005), the melting temperature of binary systems of fatty acids was lower the pure individual fatty acids. Furthermore, the melting range was narrower for pure FAME than mixtures, though the compounds melted simultaneously at one temperature when reaching the eutectic mixture ratio. The cultivars (Arlo, Polish and Echo) with medium C22:1 (23.94 to 33.04%) and medium C18:1(27.51 to 32.13%) had excellent FAME PP. It is possible that a eutectic like mixture ratio between C22:1 FAME and C18:1 FAME may occur lowering the freezing temperature of the mixtures.

It was observed that the C18:1 FAME content did not correlate with PP in Figure 3.3. This result was in agreement with Bongardt et al. (2000) and Imahara et al. (2006), who illustrated that methyl oleate content did not influence ester PP and that oil cold fluidity is determined mainly by the amount of saturated fats.

3.4.4 Fatty acid composition of *B. rapa* cv. Echo

High C22:1 rapeseed cultivars have long been a source of industrial oil. Rapeseed has been deliberately bred to produce a wide range of fatty acid profiles (Nagy & Furtan, 1978). Some early lines of rapeseed exhibit considerable genetic variability. Considerable genetic

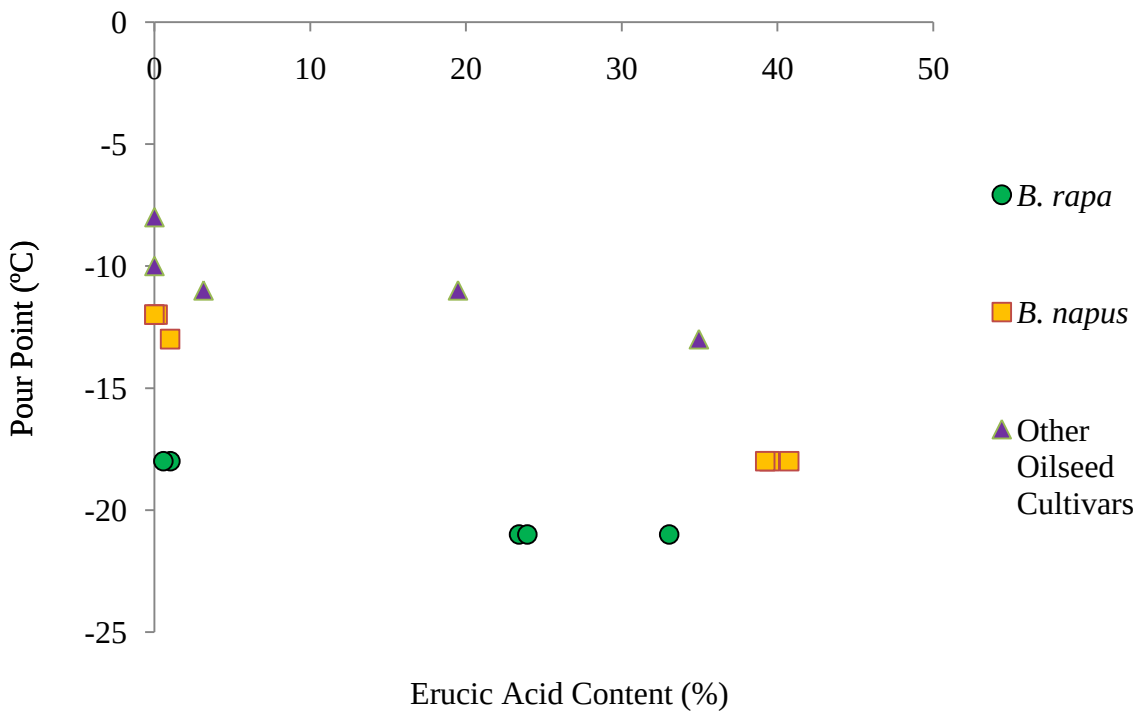


Figure 3.2 Relationship between erucic acid (C22:1) methyl esters content and pour points of methyl esters produced from five cultivars of *B. rapa* L., seven cultivars of *B. napus* L. and five cultivars from other oilseeds species (*B. juncea* L., *S. alba* L., *C. sativa* L. and *L. usitatissimum* L.)

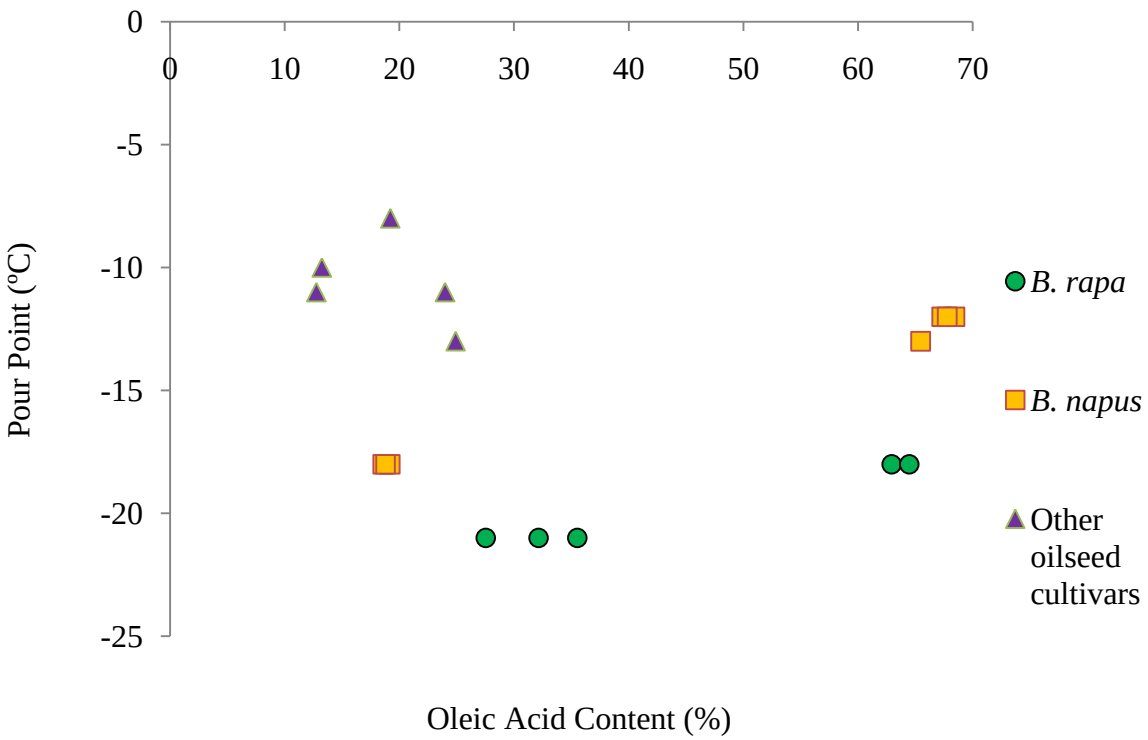


Figure 3.3 Relationship between oleic acid (C18:1) content and methyl esters pour points of methyl esters produced from five cultivars of *B. rapa* L., seven cultivars of *B. napus* L. and five cultivars from other oilseeds species (*B. juncea* L., *S. alba* L., *C. sativa* L. and *L. usitatissimum* L.)

diversity was found within *B. rapa* cv. Echo which was developed by Agriculture and Agri-Food Canada from the cultivar Polish in 1964 (Downey, et al., 1975 and Fu, et al., 2009). It was hypothesized that selection of variants in fatty acid composition within a rapeseed cultivar provides the opportunity to study the impact of TAG on oxidative stability. Seed harvested from over two thousand single plants of *B. rapa* cv. Echo were stored in individual envelopes. The fatty acid composition of seed from each single plant was determined. PUFAs of the selections ranged from 20 to 30% (Figure 3.4) while the C16:0 content was approximately 2% in all samples. Based on the low saturate content oil from individual plants of the Echo cultivar would likely have suitable low temperature behavior for use as a base oil but the oxidative stability of the oil is related to the PUFA content. The oxidative stability test requires approximately 5 mL of oil; therefore, to determine the effect of PUFA content on oil oxidative stability it was necessary to generate a larger sample of seed than was available from a single plant. To produce a suitably large sample seed from single plants with similar content of PUFA were pooled to generate larger samples of 20, 21, 22, 23, and 24% PUFA. GC analysis confirmed the fatty acid profile of these combined samples and the OSI was determined.

The fatty acid compositions of seeds pooled based on PUFA content is provided in Table 3.3. The OSI of the oils from the selected Echo plants after pooling and extraction is also provided. As expected the fatty acid composition of selected Echo seed from individual plants maintains the differences observed before pooling the samples. All of the pooled Echo seed contains less than 2.3% palmitic acid (2.1 to 2.3%). Combined C18:1 and C18:0 (35.6 to 40.2%), and C22:1 (17.9 to 20.2%) made up the bulk of the remaining fatty acids observed. The combined amount of PUFA was between 20.6 and 24.0%.

3.4.5 PUFA content and OSI

The combined C18:2 and C18:3 content of the echo seed oil was negatively correlated with OSI as shown in Figure 3.5. The OSI of oil samples with 20.6 and 24.0% PUFA was 12.6 h and 6.6 h, respectively. The data showed a trend of longer OSI as combined C18:2 and C18:3 acids concentration decreased.

This finding was consistent with previous reports that show oil stability mainly depends on the content of PUFAs (presence of double bonds) and the oxidative stability decreased with

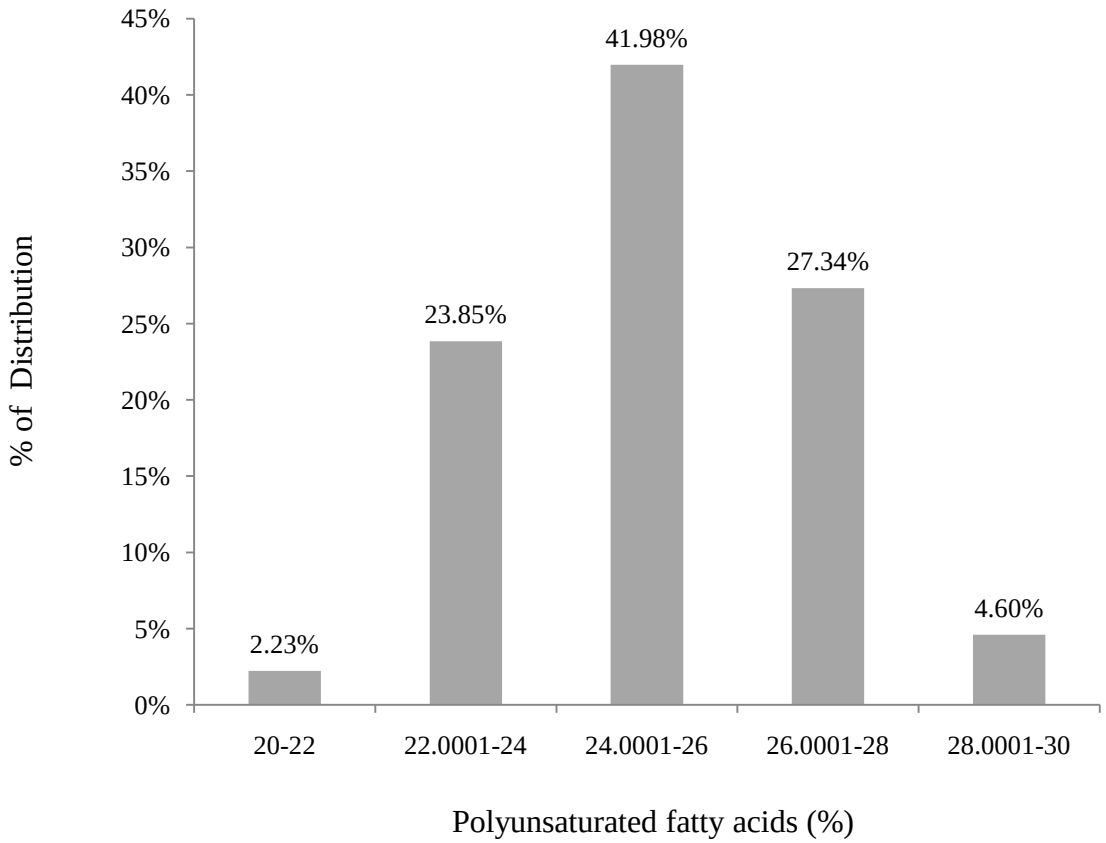


Figure 3.4 Distribution of polyunsaturated fatty acids in single plant selections of *B. rapa* cv. Echo

Table 3.3 Fatty acid profile and OSI [a] of oil obtained by hexane extraction of pooled single plant selections of *B. rapa* cv. Echo

Fatty acid (%)[b]	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
C16:0	2.31 ± 0.02	2.12 ± 0.03	2.12 ± 0.02	2.12 ± 0.02	2.05 ± 0.00
C18:0 + C18:1	35.61 ± 0.05	36.53 ± 0.08	37.91 ± 0.06	37.50 ± 0.07	40.14 ± 0.01
C18:2	16.23 ± 0.04	15.48 ± 0.04	14.89 ±0.02	14.90 ± 0.09	14.14 ± 0.02
C18:3	7.71 ± 0.01	7.31 ± 0.01	7.06 ± 0.01	6.42 ± 0.03	6.45 ± 0.03
C20:0	1.91 ± 0.01	1.98 ± 0.03	2.15 ± 0.00	2.77 ± 0.02	2.85 ± 0.07
C20:1	10.03 ± 0.03	10.35 ± 0.15	10.49 ± 0.02	8.99 ± 0.02	9.34 ± 0.01
C20:2	0.54 ± 0.01	0.50 ± 0.03	0.46 ± 0.02	0.00	0.45 ± 0.00
C22:0	4.78 ± 0.06	4.82 ± 0.03	5.08 ± 0.04	7.53 ±0.09	6.75 ± 0.07
C22:1	20.04 ± 0.01	20.17 ± 0.09	19.85 ± 0.07	19.79 ± 0.07	17.84 ± 0.07
C24:1	0.86 ± 0.01	0.76 ± 0.08	0.00	0.00	0.00
C18:2 + C18:3	23.94	22.75	21.94	21.32	20.59
Total	100.00	100.00	100.00	100.00	100.00
OSI [c]	6.60 ± 0.05	7.27 ± 0.23	8.55 ± 0.10	9.13 ± 0.10	12.57 ± 0.08

[a] Oxidative Stability Index

[b] Values are means ± standard deviations (*n*=2, n represents repeated times of analysis).

[c] Values are means ± standard deviations (*n*=3, n represents repeated times of analysis).

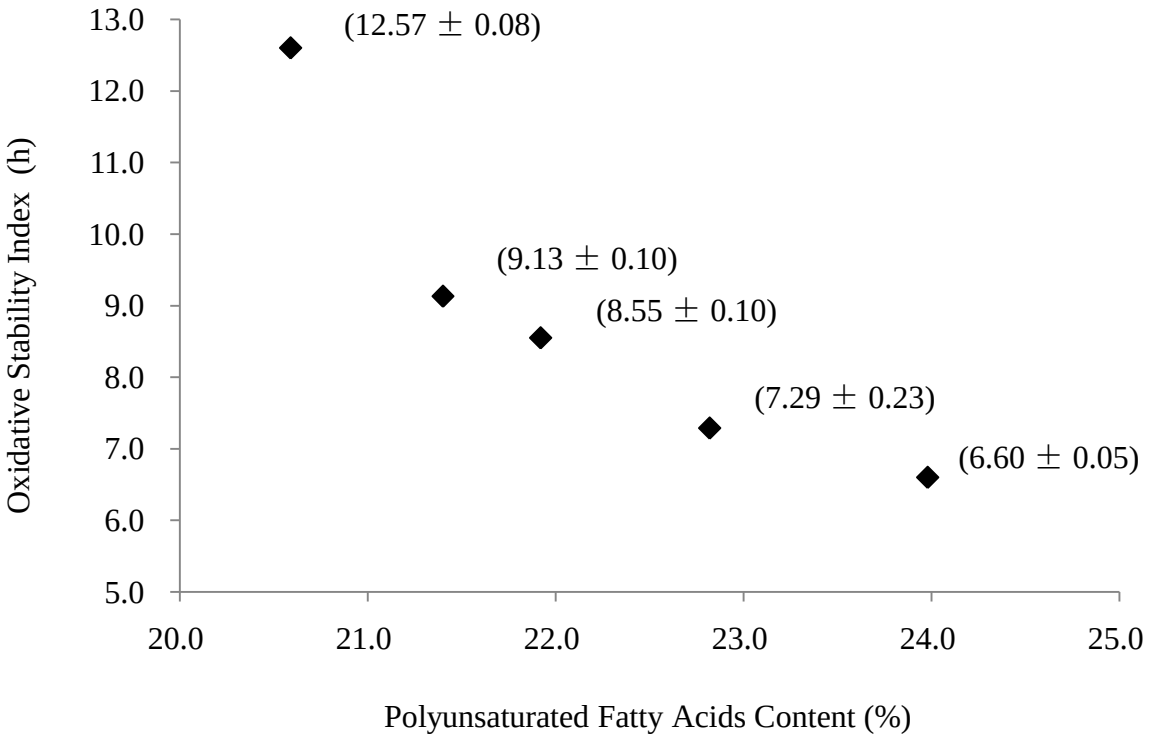


Figure 3.5 Relationship between polyunsaturated fatty acids content and Oxidative Stability Index of oil obtained from pooled single plant selections of *B. rapa* cv. Echo

the increase of the content of PUFAs (Falk, et al., 2004; Bajpai & Tyagi, 2006; Knothe, 2005). Ramo et al. (2009) noted that the position of double bonds also influenced the rate of auto oxidation. Both C18:2 and C18:3 acids, with double bonds at bis-allylic positions, are more susceptible to auto oxidation. Imahara et al. (2006) mentioned that oils with higher levels of unsaturated fatty acids produce biodiesel fuel that is more susceptible to oxidation and polymerization. These authors further suggested that fuel produced from oil with higher C18:1 content would have better stability than a fuel produced from oil with higher levels of PUFA, for C18:1 is more stable than C18:2 and C18:3. In the future an oilseed cultivar could be developed that consistently produces low levels of saturated and polyunsaturated fatty acids. A variety with less than 3.5% total saturated fatty acids and 21% PUFAs could potentially provide oil with the best reported combination of oxidative stability and low temperature flow properties.

3.5 Conclusions

In the first part of this study, seventeen oilseed cultivars from six species which had wide variations in fatty acid compositions were used to explore the correlation between fatty acid profile and oil low temperature fluidity. The results indicated that low ME PP were contributed by two factors (low C16:0 and C18:0 content and medium C22:1 content). The strong linear relationship described that low saturate content reduced the onset temperature of crystallization, thereby achieving better cold flow properties. Cultivars with medium C22:1 have lower saturate fat due to plant metabolism which may explain the correlation between medium C22:1 and excellent ME PP. It is also possible that C22:1 acid crystallization is inhibited by the presence of C18:1 FAME. Three *B. rapa* cultivars (Arlo, Polish and Echo) with less than 3.5% saturate fat and 23.41 to 33.04% C22:1content were identified with lowest observed ME PP (–21 ºC). In the second part of this study, the relationship between PUFA content and oxidative stability was investigated using *B. rapa* cv. Echo single plant selections which are genetically variable and showed with excellent low temperature properties. Good oxidative stability was observed in pooled single plant selections with low concentrations of PUFA (C18:2 and C18:3) FAME. Single plant selections with less than 20% PUFA provided the greatest stability observed. As a result, cv. Echo with both low concentration of saturate fat and PUFA content yield excellent cold flow property and oxidative stability and could be a superior feedstock for biofuel

production. This current finding requires further investigation to determine the limits for FAME properties. Ideally FAME that freezes at temperatures less than –21 ºC may be observed with lower (0 to 2.59%) saturated fat level and a higher OSI (>12.6 h) may be possible with oils with less than 20% PUFA content.

3.6 Connection to the next study

Vegetable oils are composed of TAG molecules and these molecules mainly consist of three esterified fatty acids and glycerol backbone. The main objective of this study is to formulate a vegetable oil based biolubricant with improved low temperature behavior and oxidative stability. Fatty acid profile is an important factor that influences oil physical properties. The relationship of vegetable oil fatty acid profile with oil physical and chemical properties (low temperature flow and oxidative stability) was observed.

Finally, the results showed that low temperature flow properties of *Brassica* cultivars were largely determined by saturate content. Oxidative stability of *B. rapa* cv. Echo oils was determined by polyunsaturated fatty acid content. Oils with a saturated fatty acid content of less than 3.5% and less than 20% of PUFA content have the potential to provide excellent cold temperature performance as well as oxidative stability. In this study, it was shown that certain *B. rapa* cultivars, which are low in saturate and polyunsaturated content are a superior feedstock for biofuel and biolubricant production. The data presented in this study will be used in the next studies to select the raw base oil for a superior biolubricant with good cold flow properties and oxidative stability.

CHAPTER 4
SYNTHESIS TMPESTERS BY BASE-CATALYZED TRANSESTERIFICATION

4.1 Abstract

Trimethylolpropane (TMP) [2-ethyl-2-(hydroxymethyl)-1,3-propanediol] esters were synthesized from FAMEs and TMP to produce a fluid with properties suitable for use as a lubricant base oil with good stability and low temperature performance. In this study, TAG molecules were modified to produce FAME and then linked to TMP by a two-stage base-catalyzed transesterification. In first stage, vegetable oil was transesterified with excess methanol (12% and 8% w/w, respectively) in the presence of potassium hydroxide (KOH) (twice 0.5% w/w) to produce crude FAME. After the initial step, the FAME was refined and further transesterified with TMP with heating under vacuum in a reaction catalyzed by potassium carbonate (K_2CO_3). The conversion of most of the TMP present was successfully achieved by adding an excess of FAME to a reaction mixture of base and polyol in the second step. All reactions were monitored and confirmed using ^{1}H-NMR. An efficient conversion (>96%) of FAME and TMP to TMP TE was successfully achieved by adding excess FAME slowly to a mixture of TMP and catalyst during a 2 h reaction. In conclusion, under the optimum conditions of 1 wt% catalyst, 130 ºC reaction temperature, 18 h reaction time and a mole ratio of FAME to TMP of 3.9, 100% of TMP was converted to TE.

4.2 Introduction

Vegetable oils having more 95% of TAG molecules are considered as candidates to supply low-cost, renewable and high quality base oil for biolubricant synthesis. However, both high melting point and poor oxidative stability restrict the application of vegetable oil in many lubricant applications (Fox & Stachowiak, 2007). This is, in part, due to the undesirable glycerol backbone structure in the TAG molecule. β hydrogen in glycerol backbone is an active side for elimination and oxidation reactions. As well, due to the geometry of the glycerol esters, the space

between three ester groups is minimized, thereby leading to organized packing of molecules for low steric hindrance during crystallization. Previous studies showed that the poor oxidative stability and low temperature flow properties can be significantly improved by replacing the glycerol structure by a more heat resistant branched polyol called TMP (Arbain & Salimon, 2009; Fox & Stachowiak, 2007; Erhan, et al., 2006; Campanella, et al., 2010).

Generally, TAG structure was commonly modified by transesterification reactions. Base-catalyzed transesterification reactions are favored over enzyme and acid catalyzed reactions as these catalysts achieve rapid reactions and lower production costs. Base catalyzed production of polyester of fatty acid typically requires two reaction steps. In the first step, methanolysis of TAG with alkali catalyst such as KOH is a preferred pseudo first reaction that achieves a high reaction rate. The reaction proceeds to near completion due to the low solubility of glycerol in the FAME and excess addition of methanol. In the second stage transesterification of FAME with TMP is a comparatively slow reaction that requires more heat and strong catalysts to proceed. Since transesterification is a stepwise and reversible reaction, addition of excess FAME and removing the byproduct methanol are required to shift reaction equilibrium towards the formation of TMP TE. Small-scale transesterification to produce TMP TE has been repeatedly investigated. High yields of TMP TE (85 to 99%) were synthesized by applying sodium methoxide (NaOMe) as the base catalyst (Uosukainen, et al., 1998; Yunus, et al., 2003; Gryglewicz, et al., 2003). However, when considering industrial reactions, further optimization of this second stage reaction should be considered for lowering production costs, increasing safety and improving byproduct recovery. Therefore, NaOMe is not the ideal base catalyst for many industrial applications due to its causticity, volatility and toxicity. The objective of the current study was to design of reactions for use of the lower cost and safer K_2CO_3 catalyst in the second step transesterification and optimize the reaction for higher TMP TE conversion. ^{1}H-NMR was utilized to determine reaction progress and determine product composition.

4.3 Materials and methods

4.3.1 Materials

Seeds of *B. rapa* L. and *B. napus* L. were a generous gift from Dr. K. Falk from Agriculture & Agri-Food Canada (Saskatoon, SK). Seeds of *B. juncea* L. (oriental mustard), *S.*

alba L. (yellow mustard) and *C. sativa* L. (false flax) were provided by Peacock Industries (Hague, SK), while *L. usitatissimum* L. (flax) was donated by Dr. Gordon Rowland from the Crop Development Center, University of Saskatchewan (Saskatoon, SK).

4.3.2 Oil mechanical extraction

Oilseeds were mechanically extracted using a mechanical oil press (Komet Model: CA 59G3). Crude oils obtained were filtered using a Buchner funnel lined with a glass fiber filter to remove suspended solids.

4.3.3 Free fatty acids (FFA)

Acid value is determined by AOCS official method Cd 13-60. Briefly, vegetable oil sample (1.00g) was weighed into a 250 mL Erlenmeyer flask equipped with a Teflon coated magnetic stirring bar. Subsequently, isopropanol (25 g Sigma-Aldrich, USA) and phenolphthalein indicator (2mL) were added to the flask. Contents of the flask were titrated with sodium hydroxide solution (0.1035 N; Sigma-Aldrich, USA) until the indicator turned light pink and the color persisted for a few seconds. The amount of NaOH was measured to calculate the acid value of the oil by the following Eq. [4.1]. The acid value is expressed as mg NaOH/g.

$$\text{Acid value, mg NaOH/gram} = \frac{(\text{mL of titrant})(\text{N of titrant})(40.0)}{\text{sample wt.}} \qquad [4.1]$$

4.3.4 Synthesis of FAME

The filtered oils obtained were converted to FAME using a two-stage alcoholic (methanol) transesterification process, followed by several purification steps. The oil (100g) was weighed and reacted with 12% by weight of a solution of KOH in methanol (0.5% w/w) at 22 ºC for 1.5 h. The upper phase of the reaction mixture (FAME) was reacted for the second time with 8% by weight of the same solution for additional 1.5 h. The upper phase of the second reaction was washed with water twice (3% w/w). The washed FAME layer was then dried by placing the sample in a round bottomed flask and heating the sample in a rotary evaporator (BUCHI Rotavapor R-200 system, New Castle, USA) to 75 ºC for 30 m. Later, of sodium sulphate (2 g; EMD Chemicals Inc., Germany) was added and the sample was stirred for 30 m to remove water.

After that silica gel 60 (2 g) (EMD Chemicals Inc., Germany) was added to the sample, which was stirred for 30 m to remove soap. Finally the sample was filtered through Celite 545 (EMD Chemicals Inc., Germany) producing a FAME sample that was used for the second step transesterification reaction.

4.3.5 Synthesis of TMP-based esters in one-pot reaction

As illustrated in Figure 4.1, vegetable oil FAME (81.4 g) was weighed into a tared two-neck reaction flask equipped with a magnetic stir bar and thermometer. The vegetable oil FAME was heated to about 60 ºC and then TMP (Fluka, Switzerland) (10 g) was added and melted in the flask. At this time, the base catalyst potassium carbonate (EMD Chemical Inc., Germany) (0.91 g) was added slowly into the flask. Then the mixtures were heated to the reaction temperature (130 ºC) gradually and later vacuum (28 inch Hg) was applied (model 8907A; Welch Vacuum Technology; Niles, USA) when the reaction temperature was reached. The reaction continued for 1.5 h.

4.3.6 Synthesis of TMP-based esters by reverse addition reaction

TMP (10 g) (Fluka, Switzerland) was weighed into a tared two-neck reaction flask equipped with Teflon stir bar, to which methanol was added. The TMP was stirred and dissolved in the methanol, and then 0.91 g K_2CO_3 (EMD Chemical Inc., Germany) was added to the flask. When all the compounds were well mixed, methanol was evaporated. A dropping funnel (containing 81.4 g vegetable oil methyl esters) with a sintered glass fitting was inserted in one neck of the flask. The other neck of the flask was connected to a vacuum pump (28 inch Hg). The apparatus was immersed in an oil bath and the temperature was increased initially to 60 ºC. Vacuum was applied at this temperature to remove methanol residues from the flask. Subsequently, the temperature was increased to 130 ºC and FAME in the dropping funnel was added slowly to the flask. After all the FAME was added, the reaction was continued for 2 h (shown in Figure 4.2).

4.3.7 Product characterization by ^{1}H-NMR

During each reaction the crude product was collected for analysis by ^{1}H-NMR spectrometry (500 MHz NMR equipped with TXI and BBO probe; Bruker, Bremen, Germany). Reaction samples were pipetted into NMR tubes (5 mm; Norell Standard Series, Sigma-Aldrich,

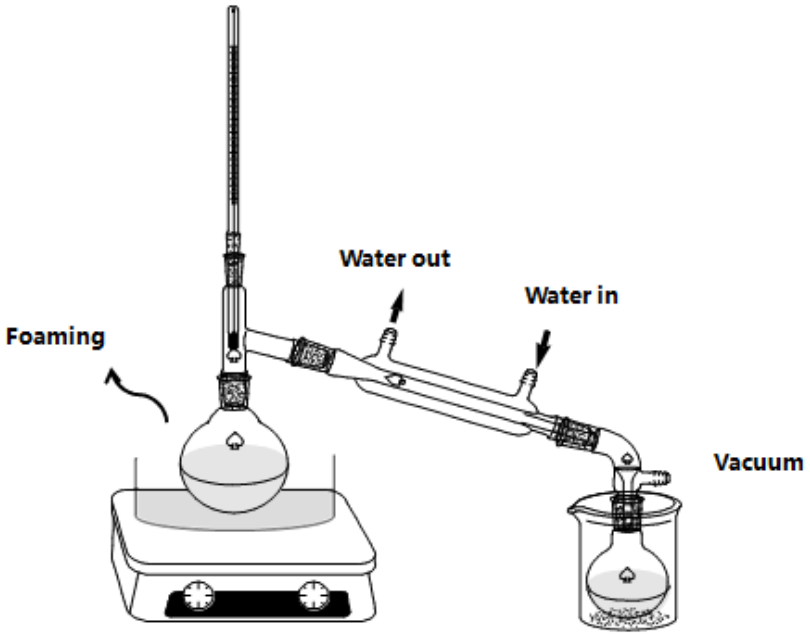


Figure 4.1 Apparatus for trimethylolpropane ester synthesis by one-pot reaction

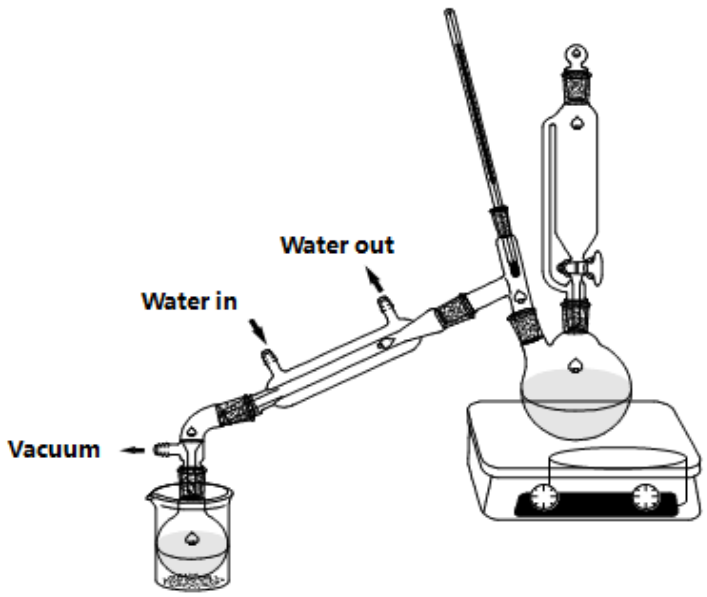


Figure 4.2 Apparatus for trimethylolpropane ester synthesis by reverse addition reaction

USA) and then deuterated chloroform ($CDCl_3$; Sigma Aldrich, Oakville Canada) was added to dissolve the low polarity esterified reaction mixtures. Where the reaction mixture was rich in partial esters deuterated methanol (MeOD) (Sigma-Aldrich, USA) was used instead of deuterated chloroform. The unprocessed free induction decay (fid) data were converted to frequency domain by Fourier transform (ACD Labs version 11). Manual baseline correction and integration was applied using the XWIN-NMR 3.0 software package (Bruker, Bremen, Germany).

4.3.8 Removal of catalyst and unreacted FAME

When the second step of transesterification was completed, the catalyst and unreacted FAME were removed from the reaction products. The reaction mixtures containing catalyst were first dissolved in hexane (Fisher Scientific, USA), and then passed through a glass column (5.2 × 28 cm) with a sintered glass bottom that was packed with celite 545 (EMD chemical Inc., Germany) to remove to residual catalyst.

Filtered eluate from the column was placed in a round bottom flask and hexane was evaporated from the sample. An aliquot of the sample was taken up in isopropanol (Sigma-Aldrich, USA) (10% w/w) for high performance liquid chromatography (HPLC) analysis. Chromatography was performed on a reversed phase column (Eclipse XDB-C18, 5 mm 4.6 × 150 mm, Agilent, USA). The mobile phase for HPLC separations was 50% acetone and 50% acetonitrile. Compounds eluted from the column in the order FAME, TMP diester (DE) and then TMP TE. Highly enriched TMP TE samples were collected for further analysis.

4.4 Results and discussion

4.4.1 Reaction monitoring by ^{1}H-NMR

^{1}H-NMR can be used as a tool to determine the environment of protons in different molecules. In strong magnetic fields protons resonate at unique radio frequencies. The molecular structure of organic compounds can often be determined by the proton signals. In this study, the reaction conversion efficiency was confirmed by ^{1}H-NMR, which provided information regarding the molecular nature of reactants and products (described from Figure 4.3 to 4.5). Protons in methylene groups which attach to the carboxylic acid ester group give signals that are diagnostic of the presence of esters in TAG, FAME and TMP TE. The glycerol protons of TAG

produce resonance peaks with chemical shifts of 4.32 ppm and 5.15 ppm (Figure 4.3). The methylene protons of methanol in a methyl ester are observed by ^{1}H-NMR resonance at 3.67 ppm (an indication of the presence of FAME; Figure 4.4). In the second reaction, the methanol methylene proton resonance disappears as methyl esters are replaced with TMP esters. These latter esters produce a characteristic resonance signal at 4.00 ppm. The migration of fatty acids during transesterification from methanol to TMP is, therefore, readily measured by ^{1}H-NMR (Figure 4.5). In addition, NMR can determine the presence of the reaction intermediate TMP DE, which produces a resonance signal at 4.02 ppm (Figure 4.6). Disappearance of this peak indicates that transesterification has neared completion.

Furthermore, integration of peak areas at 3.67 ppm and 4.00 ppm allows the quantitative estimation of the presence and concentration of unreacted excess FAME as well as partial TMP esters in the crude product. The conversion efficiency of transesterification is calculated from the integration of areas of the above two selected signals (shown at Figure 4.6) by Eq. [4.2].

$$\text{Conversion \%} = \frac{B \times 0.5 \times 0.67 + A \times 0.33 \times 1}{B \times 0.5 + A \times 0.33} = \frac{1.02B + A}{1.52B + A} \qquad [4.2]$$

Where A and B are the areas of the methylene protons at 4.02 ppm and 4.00 ppm.

4.4.2 Optimization of transesterification reactions

There were two steps in the reaction optimization process. The objective of first step is to explore the impact of catalyst type (NaOMe, K_2CO_3 and KOH) and reaction phases on base-catalyzed transesterification kinetics by using canola FAME. In the second step, the objective was to compare the conversion rates of TMP and FAME to TMP TE by applying different reaction conditions such as reaction scale, reaction time, mole ratio of FAME to TMP and source of vegetable oil FAME, in order to determine the optimum conditions for large scale transesterification. Optimized reactions would afford lower production cost as well as safe reaction conditions.

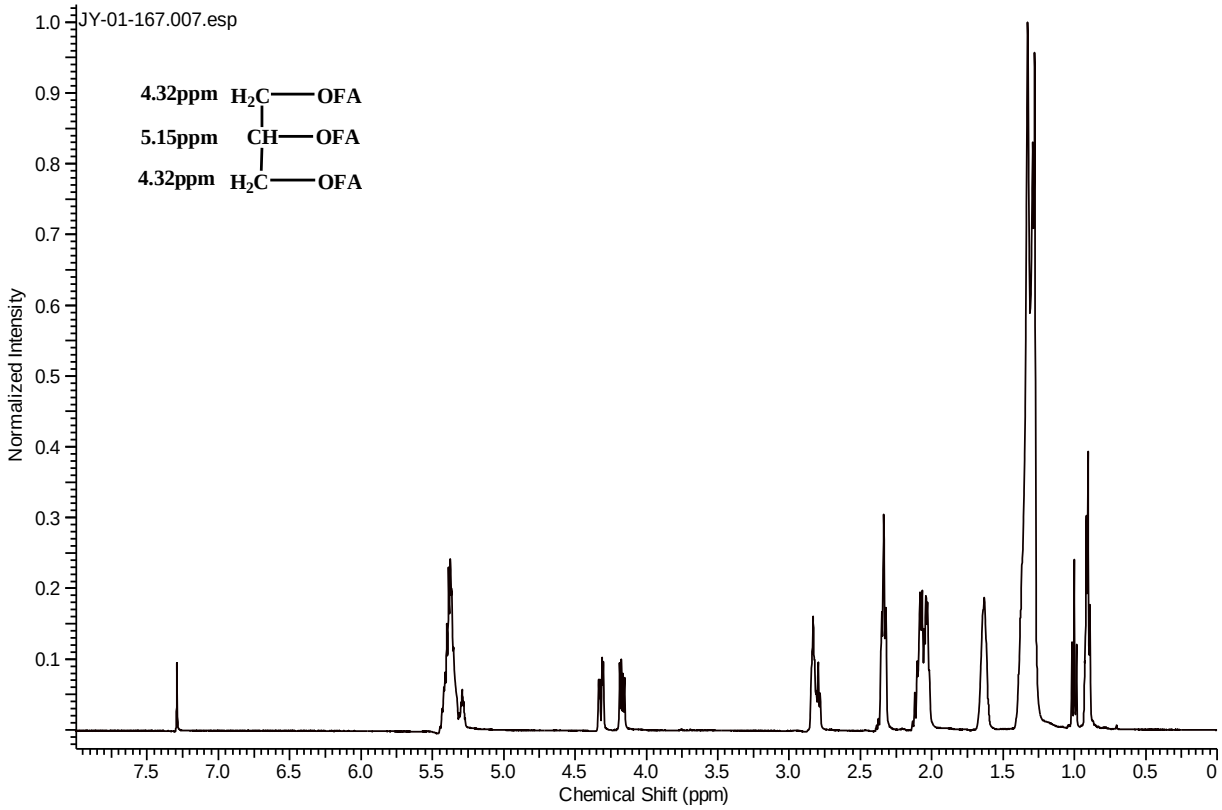


Figure 4.3 [1]H-NMR spectrum of TAG [a] molecules in camelina oil
[a] triglyceride

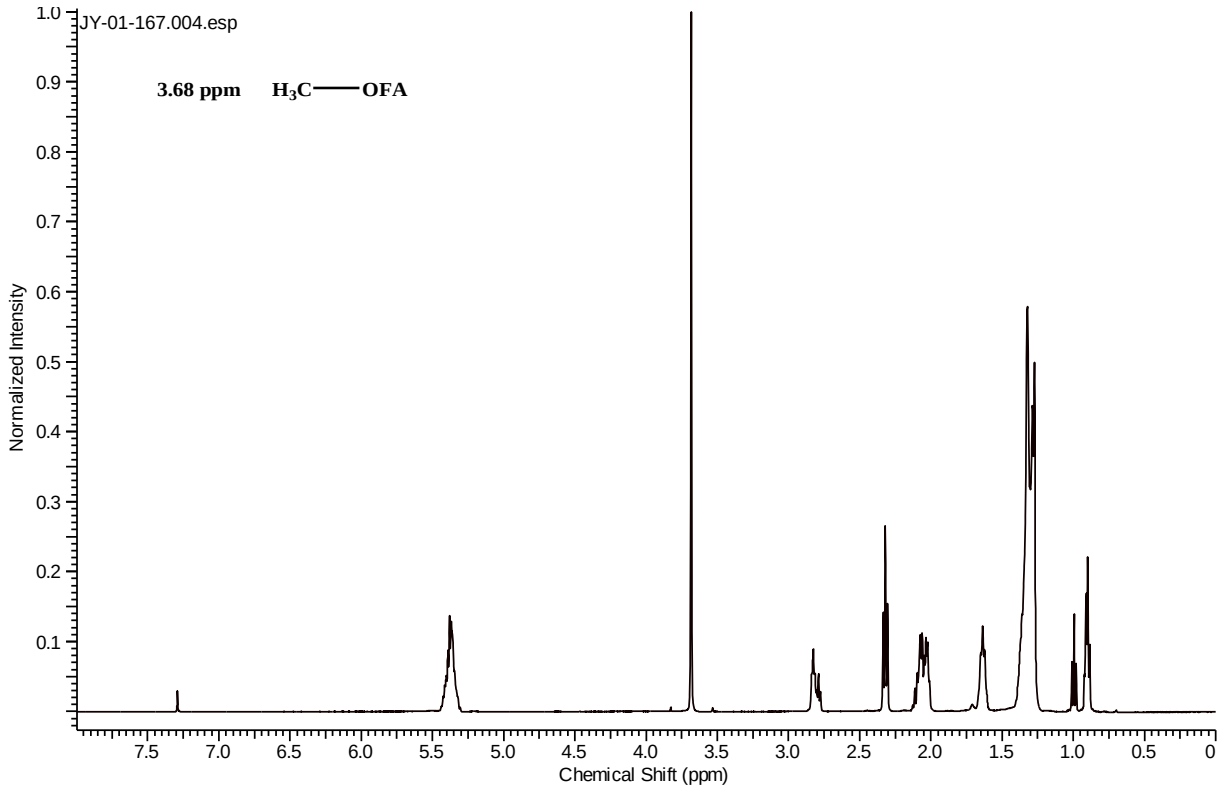


Figure 4.4 [1]H-NMR spectrum of camelina oil FAME [a]

[a] fatty acid methyl esters

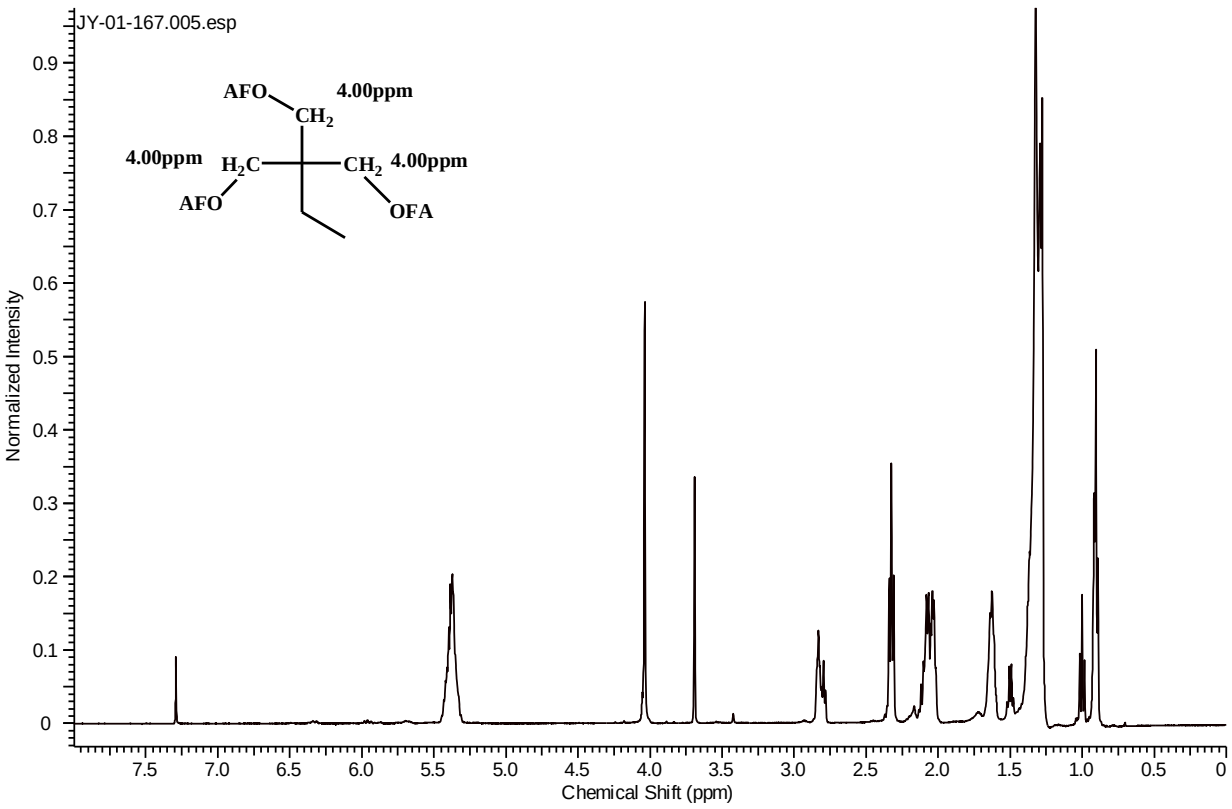


Figure 4.5 ^{1}H-NMR spectrum of camelina oil based TMP TE
[a] trimethylolpropane triesters

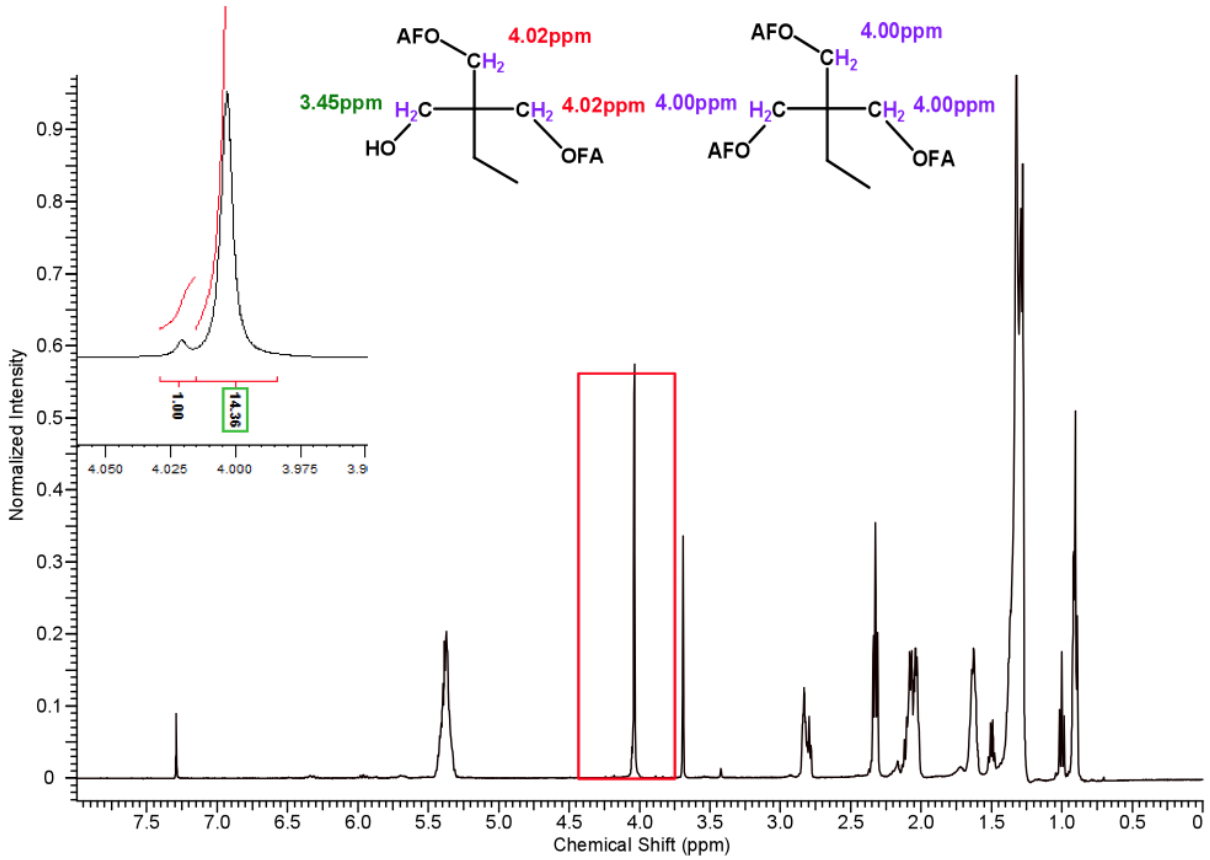


Figure 4.6 Integration of methylene protons at 4.00 ppm and 4.02 ppm represents the content of TMP TE and DE [a], respectively

[a] trimethylolpropane triesters and diesters

4.4.2.1 Reaction catalyst

Three base catalysts including NaOMe, KOH and K_2CO_3 were tested to determine impact of catalyst on reaction kinetics. As shown in Table 4.1, under the same reaction conditions (1% catalyst, 1.5 h reaction time, 3.9 mole ratio of FAME to TMP in one-pot reaction), 96.9, 95.9, and 93.4% TMP and FAME have been converted to TMP TE by using K_2CO_3, KOH and NaOMe, respectively. The results indicated that all three base catalysts achieved high conversion (95.4 ± 1.80%) in 1.5 h one-pot reactions. The results of using K_2CO_3 and KOH are comparable to previous studies. In earlier studies of methoxide catalysts the overall reaction reached completion (> 98%) in 1 h under the optimum conditions (Yunus, et al., 2003). Earlier reports indicate conversion with this catalyst in10 h (Uosukainen, et al., 1998) and 20 h as well (Gryglewicz, et al., 2003). As these three base catalysts produced similar conversion the safer and less catalysts K_2CO_3 and KOH would be preferred for industrial synthesis. If the reaction conditions could be further optimized, it is possible to achieve higher conversion rate.

4.4.2.2 Reaction phase

In one-pot reactions, canola FAME was mixed vigorously in the reaction flask containing all reagents including TMP and catalyst. The reaction mixture was added to a cold reactor and heated, reaching the reaction temperature after several minutes of heating. Previous literature showed that 85 to 99% of TMP TEs were synthesized in one-pot reactions using sodium methoxide and calcium methoxide (Gryglewicz, et al., 2003; Yunus, et al., 2003; Yunus, et al., 2005; Uosukainen, et al., 1998). In this study, 95.4 ± 1.8% TMP TEs were converted using NaOMe, KOH and K_2CO_3. Although high conversion rate could be achieved using a one-pot reaction, the reaction foamed excessively due to the rapid loss of methanol. When all the reactants are mixed at the beginning of the reaction and the reaction rate was rapid, large amounts of methanol vapour were released under vacuum. This finding was also consistent with earlier studies. In order to contain the reaction the vacuum may be applied gradually (Yunus et al., 2003). Unosukainen et al. (1998) heated the reaction mixture to 85 ºC and vacuum was only applied when temperature reached 110 ºC in order to control foaming. One-pot reactions are not ideal for larger scale reactions as it is difficult to recover the methanol without prevention of foaming.

In an alternate approach called a reverse addition reaction, FAME was added slowly to the mixture of TMP and catalyst at the reaction temperature. This technique of adding FAME slowly

Table 4.1 Conversion of canola TMP TE[a] in different reaction conditions

Catalyst	Reaction time(h)	Phase	Mole ratio of FAME[b] to TMP [c]	Conversion (%)
NaOMe	1.5	One-pot	3.9	93.4
KOH	1.5	One-pot	3.9	95.9
K_2CO_3	1.5	One-pot	3.9	96.9
K_2CO_3	2	Reverse	3.3	98.5
K_2CO_3	2	Reverse	3.9	99.2
K_2CO_3	18	Reverse	3.9	100
KOH	18	Reverse	3.9	100

[a] trimethylolpropane triesters
[b] fatty acid methyl esters
[c] trimethylolpropane

into the reaction system at the reaction temperature, where other conditions were similar to one-pot reactions produced 99.2% of TMP TEs in a 2 h reaction. Quantitative conversion of TMP to TEs was achieved in 18 h by this method. Moreover, the methanol foaming problem was solved by slow addition of FAME. The small amount of methanol formed during Fame addition was continuously removed by the vacuum pump. The finding was further supported by US patent 4,517,360, which describes an alternate approach to controlling esterification reactions that produce foam. In the patent, excess fatty acid polyesters were slowly added into the mixture of polyol, fatty acid esters and catalyst. Foaming problems were successfully solved by the reverse addition reaction. In summary, reverse addition transesterification not only solves the foaming problem, but also achieves a high conversion rate (98.5 to 100%) in this study. In the following step, the optimization of reverse addition K_2CO_3 catalyzed transesterification will be explored.

4.4.2.3 Reverse addition transesterification optimization

The conversion rates of K_2CO_3 catalyzed reverse addition transesterification under different conditions including oil source, reaction scale, reaction time, mole ratio of FAME to TMP were studied (Table 4.2). Conditions for one-pot NaOMe catalyzed transesterification (98%) were optimized by Yunus, et al. (2003; 130 ºC reaction temperature, 3.9 mole ratio of FAME to TMP, 29 mbar pressure and 0.9% catalyst).

The impact of mole ratio of FAME to TMP in reverse addition reactions has been evaluated. The stoichiometric ratio for transesterification requires three moles of FAME and one mole of TMP to produce one mole of TMP TEs and three moles of methanol. The mole ratio of FAME to TMP was varied between 3.0 and 3.9 while other conditions were held constant (1% K_2CO_3, 130 ºC reaction temperature, 2 h reaction time, 28 inch Hg vacuum). Total conversion without excess FAME (90.7 ± 1.7%) was lower than that with 30% excess FAME (94.7 ± 2.1%). This result illustrated that a higher mole ratio of FAME to TMP shifted reaction equilibrium to the forward reaction and can suppress the reverse reaction, which was in agreement with the study by Kamil and Yusup (2011). Kamil and Yusup (2011) demonstrated that polyol esters conversion increased when the mole ratio of FAME to TMP was increased from 2.5:1 to 4:1, and the rate stayed constant when a higher ratio (7.5:1) was used. Therefore, excess FAME was required to drive the reaction to completion; however, a 3.9:1 mole ratio is preferred for economical consideration and the removal of excess FAME after the reaction.

Table 4.2 TMP TE [a] conversion of different vegetable oils using K_2CO_3

FA[b] Source	Scale	Ratio of FAME [c] /TMP [d]	Reaction Time (h)	Conv.[e] (%)
Canola	S	3	2	92.4
Yellow mustard	S	3	2	89.0
Oriental mustard	S	3	2	90.7
Canola	S	3.9	2	93.2
Oriental mustard	S	3.9	2	96.1
Canola	L	3.9	2	97.7
Corn	L	3.9	2	97.9
Flax	L	3.9	2	96.9
Canola	L	3.9	18	100
Corn	L	3.9	18	99.7
Flax	L	3.9	18	97.6
Rapeseed	L	3.9	18	97.3

[a] trimethylolpropane
[b] fatty acids
[c] fatty acid methyl esters
[d] trimethylolpropane
[e] conversion

The reaction duration was also investigated in this study. The reaction duration was calculated from the time when all of the FAME was added to the reaction flask. High conversion (96.4 ± 1.9%) was achieved in 2 h reactions off our different vegetable oils reacted with K_2CO_3 (1%) at 130 ºC and 28 inch Hg vacuum. Conversion reached 99.1 ± 1.3% after 18 h under the same conditions. An apparent 100% conversion was achieved while reacting canola oil esters under these conditions. The results generally agreed with earlier studies where the formation of TE begins after just 5 to 10 m. While the reaction rate is slow at first the reaction proceeds quickly and reaches equilibrium in 20 m (Yunus, et al., 2005). However, when the FAME is added slowly it is not easy to calculate the reaction rate before all the FAME is added to the reaction flask. In this study, 85% of TEs were present immediately after adding all the FAME. The conversion increased slowly afterwards as 96.4% conversion was observed after 2 h while apparent 99.1% conversion required a 18 h of reaction for the four vegetable oils. Although about 3% higher conversion can be achieved by the longer reaction time a 2 h reaction is preferred to limit costs related to energy and labour while increasing reaction yield.

FAME derived from six vegetable oils was converted to TMP esters using the optimum conditions developed in this study. Reaction scale was found to have a significant influence on the conversion rate under the same conditions. For 20 g of total reactants 92.3 ± 2.7% TMP TEs were produced, while for 100 g of total reactants 98.2 ± 1.2% TMP TEs were produced. Moreover, only the larger scale reaction yielded 100% apparent TE yield from canola oil in the18 h reaction. It is possible that the vacuum applied in the smaller scale reaction was not as good as that of the larger scale reaction. Also, the larger scale reaction provided more surface contact among the reactants. Under the same reaction conditions, 100%, 99.7%, 97.6% and 97.3% conversion rates were approached by using different vegetable oils including canola oil, corn oil, flax oil and rapeseed oil, respectively.

TMP TEs were successfully synthesized at near quantitative yield (96.4%) using K_2CO_3 as base catalyst in a two-step transesterification reaction under the following optimum conditions: Mole ratio of 3.9 moles FAME to 1.0 mole TMP containing 1% w/w K_2CO_3 catalyst, then holding the reaction products for 2 h at 130 ºC and 28 mm Hg vacuum pressure.

4.4.3 Separation of TMP TE with FAME in crude product

Reversed phase chromatography allows the separation of compounds based on their polarity. In reversed phase chromatography the interaction between solute molecules of different polarities dissolved in a chromatographic mobile phase and a stationary phase are used to effect a separation. The crude product which has been dissolved in isopropanol (10% solution) was injected and passed over a C18-reversed phase HPLC column. TMP TE and FAME in crude products could be separated on the C18-reversed phase HPLC column based on differences in polarity. FAME, the more polar compound, was eluted within the first three minutes, while TMP TE, a comparatively less polar compound, was eluted between 13 and 70 m (shown in Figure 4.7). Therefore, the preparative reversed phase HPLC column separated FAME and TMP TE. It is possible that HPLC could be used to enrich the TMP TE for subsequent analysis. However, this method was not applicable for large-scale separations; thus, only small amount of pure TMP TE could be obtained. In addition a large amount of solvent is needed to achieve the separation. This solvent must be evaporated from the sample before it. This approach may not be practical for commercial purposes but HPLC can be used as an analytical tool.

Compared with previous investigation, molecular distillation was the most common method used to remove excess FAME from TMP esters (Yunus, et al., 2003). Although molecular distillation could purify the product, the high temperature distillation may cause damage during distillation of the crude product; for example, double bonds of the PUFA might undergo conjugation during high temperature distillation.

Based on the above chromatographic method pure samples of TMP esters from different vegetable oils could be separated from FAME. The next chapter involves the characterization of the TMP based biolubricant by determining the T2 relaxation of a ^{1}H-NMRresonance signal at 4.00 ppm arising from TMP TE.

4.5 Conclusions

The aim of this study was to develop and optimize the second stage of transesterification of FAME and TMP with lower cost base catalyst while overcoming some critical problems (such as foaming and safety handling of catalyst) in large scale reactions. This study showed the benefits of applying reverse addition transesterification in the second stage of the reaction. ^{1}H-NMR has been successfully utilized for rapid analysis of reaction products as well as

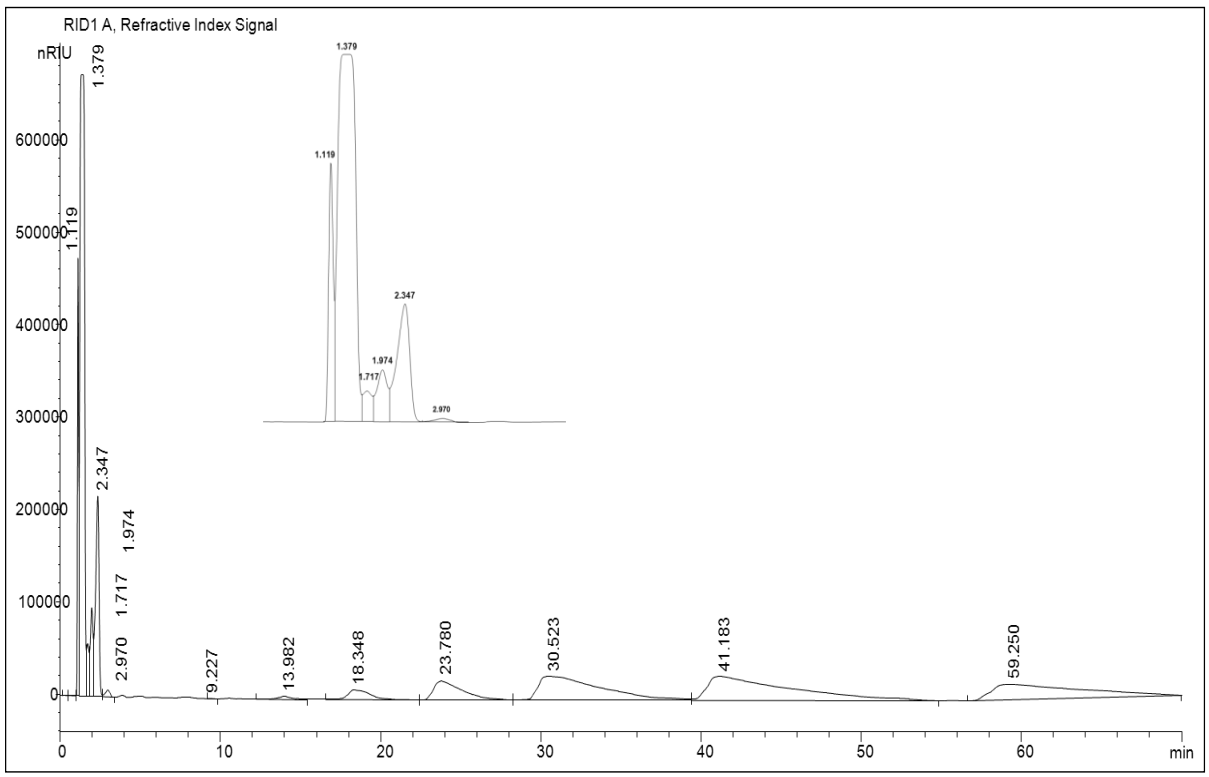


Figure 4.7 HPLC chromatogram of crude product including TMP TE[a] and excess FAME[b]

[a] trimethylolpropane triesters

[b] fatty acid methyl esters

monitoring reaction progress. The data indicate that both K_2CO_3 and KOH are alternative catalysts that may be used in place of NaOMe. In addition reverse addition was more effective than the one-pot reaction in polyester synthesis. The optimum conditions of reverse addition reactions were tested on six vegetable oils and the method was successful in generating high yields of esters with all oils. Finally, the optimum conditions of those tested were selected for reaction speed where TMP was efficiently converted (96.4%) to TMP TE with K_2CO_3 catalyst (1% of w/w K_2CO_3 catalyst, 2 h reaction time, 3.9 mole ratio of FAME to TMP, 130 ºC reaction temperature and 28 mm Hg high vacuum).

4.6 Connection to the next study

The previous two chapters described 1) identifying optimum oil for production of stable base oil with superior low temperature flow properties and 2) methods to convert oil to a TMP based biolubricant. In the next study we continue elucidation of TMP based biolubricant from vegetable oils produced with potassium carbonate and potassium hydroxide. In this chapter, the major objective was to develop a method and optimize reaction conditions for TMP based biolubricant production. By utilizing a reverse addition reaction 96.4% of TMP was converted to biolubricant (1% of w/w K_2CO_3 catalyst, 2 h reaction time, 3.9 mole ratio of FAME to TMP, 130 ºC reaction temperature and 28 mm Hg high vacuum). It was proposed that the reaction products be separated by C18 reversed phase HPLC column chromatography. The highly enriched TMP biolubricant prepared by chromatography was to be characterized to determine both OSI and low temperature performance in the next study. The above optimum conditions were applied to different vegetable oils. In the next chapter the physical and chemical properties of the products synthesized in this study will be reported. Results of the HPLC separation were not satisfactory when the sample was observed by ^{1}H-NMR. From the spectrum, new peaks were observed that did not belong to the TMP esters sample. While contamination from the HPLC column or the solvents is possible the presence of these compounds would render future studies of the TMP esters purified by HPLC suspect. Therefore, the next study was performed on samples that contained both TMP TE and unreacted FAME that were used without further separation.

CHAPTER 5
CHARACTERIZATION OF TRIMETHYLOLPROPANE BASED BIOLUBRICANT

5.1 Abstract

The Oxidative Stability Index (OSI) of TMP esters produced from five vegetable oils (*B. rapa* L., *L. usitatissimum* L., *Zea mays* L., *B. napus* L., *C. sativa* L.) were determined. The OSI of original vegetable oils and FAMEs were also measured as reference. Results showedthe highest stability was observed in vegetable oils while the processed products were less stable.Excess FAME in the crude product and the lost of natural antioxidants by refining of the reaction products with silica and celite column were determined to be the major causes of the loss of oxidative stability. The low temperature flow properties of TMP esters produced from four different vegetable oils (oriental mustard, flax, rapeseed and camelina) were investigated by ^{1}H-NMR. The T2 relaxations of different TMP esters were measured to observe how the mobility of oil change at decreasing temperature intervals until the singlet at 4.2 ppm disappeared. Generally, increase oil mobility (represented by T2) is linearly correlative with rising temperature. The Gaussian widths of the singlet in each oil described that the mobility of the molecules until 248 K was still good.

5.2 Introduction

Lubricant oils are made up of inert base oil and additives that alter oil properties to meet the needs of the lubricant. Most lubricant base oils are refined from lower cost crude petroleum feedstocks. However, environmental pollution as well as renewability are driving the development of synthetic esters from vegetable oils which are readily available and environmentally friendly (Kamil et al., 2011). Producing vegetable oil based synthetic esters can significantly improve vegetable oil thermal and oxidative stability as well as cold flow properties. Gryglewicz et al. (2003) demonstrated that TMP esters of fatty acids gave higher resistance to oxidation than TAG esters. The effect of replacing the glycerol backbone from TAG

by TMP on low temperature behaviors was also investigated in earlier papers. The PP of TMP esters produced from *Jatropha curcas* oil was –30 ºC, while the original oil PP was 10 ºC (Arbain & Salimon, 2010). It was also shown that NPG and TMP esters produced from lard (PP at 32.3 ºC) had PP of –13.5 and –10.5 ºC, respectively (Gryglewicz et al., 2003).

Traditionally, PP test was used to study and predict the oil mobility and operability in low temperature conditions. PP test requires about 75 g of oil for each sample for triplicate tests. Recently, some studies applied ^{1}H-NMR as a non-destructive analytical tool to characterize compound molecular mobility by measuring the transverse relaxation (T2) (Sun & Isayev, 2007; Urahama, 2010). Relaxation analysis of proton NMR signals provides important information about the chemical environment of a molecule. For example, short transverse relaxation (T2) arise from solid molecules, for they pack tightly and will not slide or move, while long T2 comes from flexible molecule such as liquids in which molecules are farther apart.

In this study, the OSI of TMP of base biolubricant was measured by oxidative stability instrument and relaxation time experiments were conducted to investigate to observe how the mobility of TMP esters change in decreasing temperature intervals (298 K, 288 K, 278 K, 273 K, 268 K, 263 K…) until the singlet at 4.2 ppm disappeared. Relaxation (T2) of TMP esters was measured by Voight function single peak fitting.

5.3 Materials and methods

5.3.1 OSI measurement

The OSI was measured according to American Oil Chemist Society (AOCS) method cd 12b-92 using an oxidative stability instrument (Omnion, Inc. Rockland, MA). Crude TMP based esters (vegetable oil and vegetable FAME) were bubbled with nitrogen to minimize contact with air and stored at –4 ºC fridge before OSI measurement. Different samples (5.0 ± 0.2 g) were weight directly to the reaction tubes. Then deionized water (50.0 mL) was added into the conductivity tubes and the conductivity probes were inserted. The initial conductivity reading was less than 950 units. The air pressure was set to 5.5 psi and the heating block was adjusted to produce 110 ºC. Samples were inserted in the heating block and the tubing was connected from the air manifold to the conductivity measurement tube then airflow was started. Conductivity electrodes connected to a computer were used to monitor the current in each cell every three

minutes (24 channels OSI). The measurement is complete after a rapid increase in conductivity signaling accumulation of organic acids. The time period between the start of analysis and the induction point is the OSI.

5.3.2 Low temperature mobility tested by ^{1}H-NMR

Purified samples were added to a NMR tube (1 mm diameter) (NI5CCI-B, Norell Inc., Landisville, NJ) (shown in Figure 5.1) and then the narrow tube was placed in a larger NMR tube (5 mm outer diameter) containing deuterochloroform ($CDCl_3$) (Sigma Aldrich, Oakville Canada) as an external reference. Sample was introduced to the NMR spectrometer using ceramic spinner. ^{1}H spectra were accumulated at room temperature (298 K). Subsequently the temperature control probe was adjusted to a series of temperatures (298 K, 288 K, 278 K, 273 K, 268 K, 263 K, 258 K, 253 K, 248 K, 243 K, and 238 K) by increasing gas flow (nitrogen) to 500 L/h. When reaching the set temperature the sample was maintained at the test temperature for 5 m before recording the spectrum. After each analysis, the unprocessed free induction decay (fid) data were then converted frequency domain by Fourier transform (MestReC Lite, Mestrelab Research SL, RegistroMercantil, Spain).Samples were collected at temperatures where the singlet at 4.2 ppm was readily discerned from the baseline (illustrated in Figure 5.2). Frequency domain spectra were processed to extract using the region at 4.2 ppm (Origin Lab Cooperation, Northampton, MA01060, USA) for further analysis. The singlet at 4.2 ppm from each spectrum was fitted to a Voigt function. The measurement of the Gausian width, Lorentzian width, peak area and peak intensity of NMR signals in a TMP ester molecule was used as to provide a measurement of the physical environment of molecules in the sample.

5.4 Results and discussion

5.4.1 Oxidative stability

The OSI value of original vegetable oils, their FAME and TMP esters dropped significantly (varying from 29.7 to 54.9% decreases) after the first step of transesterification (Table 5.1). The OSI of flax FAME was not measurable as the large amount of polyunsaturated acid led to rapid oxidation that could not be characterized by the OSI instrument. In all cases FAME was less stable than its parent vegetable oil. The FAME is a smaller molecule, only one

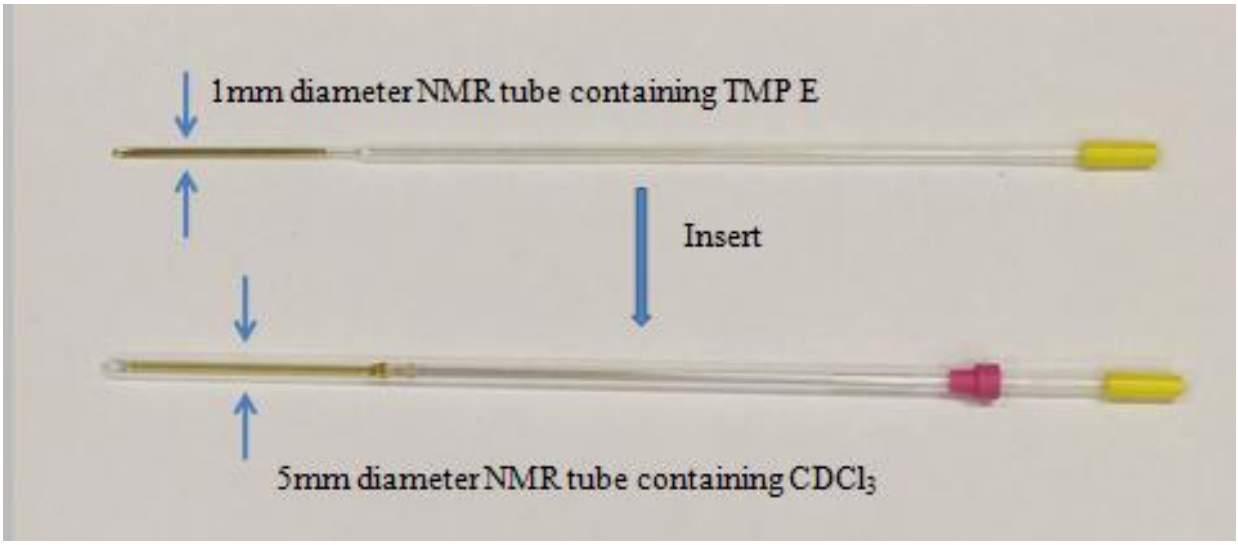


Figure 5.1 Structure of the special NMR sample tube used for low temperature fluidity analysis

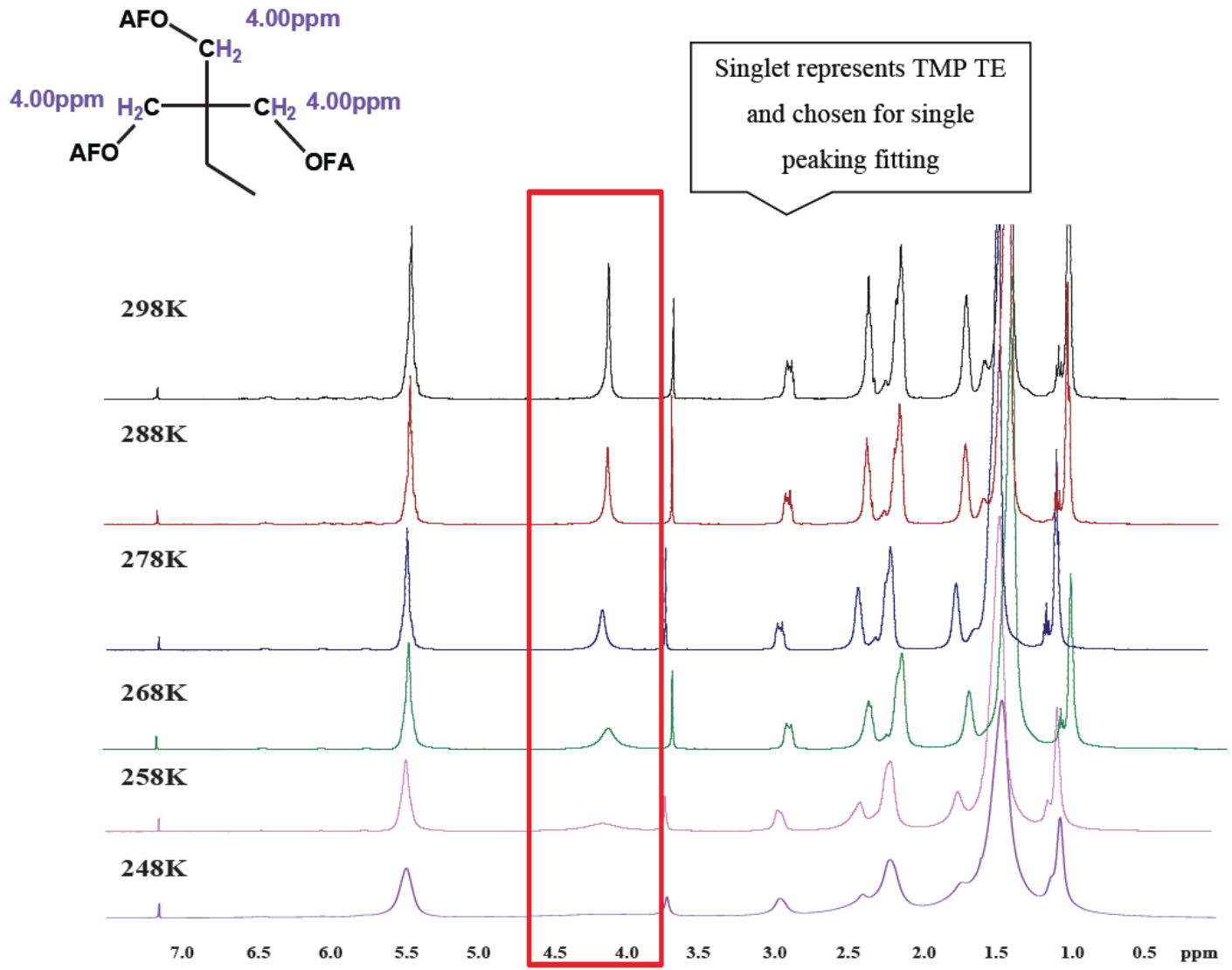


Figure 5.2 ^{1}H-NMR spectra for camelina oil at temperatures between 298 K and 248 K

third of TAG molecule, and less viscous. The lower viscosity may contribute some of the observed decrease in OSI. Similar results were reported for the OSI value of meadowfoam oil (67.3 h at 110 ^{o}C), which decreased significantly when it was transesterified to produce meadowfoam FAME (4.7 h at 90 ^{o}C) (Isbell, et al., 1999).

It was observed that the OSI of TMP esters was more variable and smaller than their parent vegetable oils (Table 5.1). The lower stability may have been due to the presence of excess FAME (30%) present in the crude product, which may influence the OSI of the sample. As mentioned above, the oxidative stability of FAME was much less than the parent vegetable oil. TMP ester and FAME stability might be also be decreased by contact with strong base at high temperature during the two-transesterification steps. The crude sample was dissolved in hexane and passed through the celite 545 column in order to remove the soap and catalyst after the reaction. The celite column may have removed antioxidants, which exists in natural vegetable oils, during contact with the oil. Therefore celite 545 refiningmay have influenced the OSI value. In previous studies, the crude soybean and peanut oils had much greater OSI value than their refined RBD counterparts (Dunn, 2005). This was attributed to the loss of some natural antioxidants in vegetable oils during refining and processing. Crude vegetable oils contain natural antioxidants such as tocopherols, which can protect the oil from oxidation (Akoh, 1994). Therefore, a study of the effect of celite refining on stability was undertaken. Corn oil, its FAME and TMP esters were passed over a silica gel 60 column. The result of the absorbent treatments is provided in Table 5.2.

The OSI value of refined corn oil samples was 4.6 h while unrefined samples had an OSI value of 7.2 h. Using the same treatment with FAME OSI decreased from 3.7 to 2.0 h while for the TMP esters OSI decreased from 7.2 to 2.7 h with refining. Therefore, a refining process that utilizes silica and celite absorbents may significantly decrease the OSI value of samples. The crude TMP esters sample showed the greatest loss of OSI with refining. Therefore, the loss of natural antioxidants during refining may have a major impact on OSI. However, the OSI the parent corn oil and its TMP esters are the same (7.2 h). This result may indicated that the oxidative stability of oil was improved after modification to form TMP esters as the crude TMP esters still contained 30% unreacted FAME which has low OSI value.

Table 5.1 OSI [a] of different vegetable oils, their FAME [b] and TMP TE [c]

Source	Oil (h)	FAME [b] (h)	TMP TE (h)
B. rapa	12.6±0.1	6.1±0.2	6.3±0.1
L. usitatissimum	1.4±0.2	N/A	1.4±0.2
Z. mays	7.2±0.1	3.7±0.2	2.7±0.1
B. napus	8.2±0.2	3.7±0.1	5.5±0.2
C. sativa	3.7±0.3	2.6±0.3	1.1±0.1

[a] Oxidative Stability Index
[b] fatty acid methyl esters
[c] trimethylolpropane triesters

Table 5.2 OSI [a] of corn oil and its FAME [b] and TMP TE [c] with and without column filtration

Sample	OSI of control (h)	OSI of sample (Silica and celite column) (h)
Oil	7.2	4.6
FAME	3.7	2.0
TMP TE	7.2	2.7

[a] oxidative stability index
[b] fatty acid methyl esters
[c] trimethylolpropane triesters

5.4.2 Low temperature mobility of TMP esters

The shape of spectral lines of singlet peaks observed by ^{1}H-spectrometry is best described by a Lorentzian function, however, when the magnetic field is inhomogeneous, the shape of singlet peaks may become more Gaussian. The Voigt peak was developed to analyze spectral peaks that have both Lorentzian and Gaussian characteristics. The molecules of TMP esters may exist in multiple states during exposure to low temperatures where crystallization may occur. At warm temperatures the molecules were in a liquid phase with good fluidity. Then later the sample started to become cloudy. In this state it was a gel with both liquid and solids present at the same time. If the temperature is lowered sufficiently the oil may form a solid where the molecules of the oil have poor mobility. Solid materials may not have the same magnetic susceptibility as liquids. When this happens the magnetic field in the NMR may lose homogeneity. As the loss of homogeneity is a random effect the line shape of peaks from samples may be broadened randomly generating a Gaussian component to the line shape. Therefore, the spectral lines generated from cooling samples may be studied to determine the contribution of Lorentzian and Gaussian broadening of peaks. The Voigt function is a line shape derived by the convolution of both Gaussian profile and Lorentzian equations. The data obtained from the Voigt function single peak fitting of oriental mustard TMP TE at temperatures from 298 to 248 K is presented in Table 5.3 including all equation parameters (y_0, A, Xc, wL and wG) of the TMP TE singlet. All the peaks analyzed were well fitted by the Voigt function (R^2>0.999).

It was observed that the Gaussian widths of the oriental mustard oil TMP TE singlet from 298 K to 258 K were small, varying between 3.96×10^{-13} and 2.2×10^{-7} (Table 5.3).The small contribution of Gaussian shape to the spectral line indicate that the magnetic homogeneity of the NMR was not substantially affected by temperatures above 258 K.TMP TE of oriental mustard oil just slightly changed and the sample was probably in a gel form, which contained mostly liquid. Under 258K (–15 ^{o}C), the TMP biolubricant still showed excellent fluidity. However, when the temperature dropped down to 248 K, the Gaussian width significantly increased to 0.33. This existence of a larger contribution of Gaussian to the fitted curve illustrated the possibility that larger amount of solid particles may have formed in the sample thus increasing magnetic inhomogeneity. A similar finding was observed in other vegetable oils studied (camelina, rapeseed and flax). The Gaussian widths of TMP TE singlet in different oils were small and showed little variability at 258 K and warmer. However, when the temperature was 248 K and

Table 5.3 Voigt fitting parameters of oriental mustard TMP TE [a] between 298 K and 248 K

No. of sample	147.2	147.3	147.4	147.5	147.6	147.7	147.8	147.9
Temperature (K)	298	288	278	273	268	263	258	248
y_0 [b]	−0.4693	−0.62108	−0.74675	−0.63254	−0.36406	−0.20882	−0.88311	0.29221
Xc [c]	4.26789	4.27788	4.28685	4.29123	4.29605	4.29956	4.29699	4.30095
A [d]	3.83793	4.14169	4.37083	4.47086	4.51529	4.4936	4.453	2.97799
wG [e]	3.96E-13	2.31E-08	8.83E-09	3.83E-08	3.86E-08	1.48E-08	2.20E-07	0.33271
wL [f]	0.02016	0.03003	0.0515	0.07178	0.10442	0.16033	0.26183	0.53578
R square	0.999	0.99976	0.99984	0.9999	0.99991	0.99988	0.99985	0.99959

[a] trimethylolpropane triesters
[b] distance between singlet baseline and X axis
[c] center of singlet (expressed by ppm)
[d] singlet area
[e] Gausian width
[f] Lorentzian width

lower, the Gaussian width increased significantly in TMP TE derived from camelina, oriental mustard and flax. The width of spectral peaks of TMP esters produced from rapeseed oil (*B. rapa* cv. Echo), which was proven with excellent low flow property in Chapter 3, did not increase as much. The results indicated that rapeseed oil had superior low temperature fluidity properties at temperatures below 248 K. According to the Gaussian widths TMP TE derived from flax oil produced the next greatest fluidity followed by camelina oil and oriental mustard oil.

Compared with Gaussian component of NMR singlet width, the Lorentzian component width contributed a much higher portion of the observed peak width. At 298 K, the singlet was sharp and narrow (described in Figure 5.2). With decreasing temperature (from 298 to 248K) the peak became shorter and wider but maintained a basically Lorentzian shape. The Lorentzian width at half peak height of the NMR peaks is also known as the T2 (spin-spin relaxation time).T2 is a time constant that is proportional to the rate of NMR signal decay and inversely proportional to peak width. As the temperature decreased the chemical environment of TMP TE protons changed resulting in decreased relaxation times. There were strong linear correlation between ln(T2) and temperature (R^2 ranged from 0.945 to 0.985 in four vegetable oils). T2 decreased with decreasing the temperature suggesting decreased molecular motion. T2 measurements taken by ^{1}H-NMR have also been reported to enable the measurement of mobility in wheat starch (Choi & Kerr, 2003). The equations describing the relationship between T2 and temperature of four TMP TE derived from vegetable oils are provided as Table 5.3. Flax oil had smallest slope and a longer relaxation time than other oils from 298 K to 248 K. As flax oil contains large amounts of polyunsaturated fat (about 80% combined linoleic and linolenic acids) and the melting points of these fats are lower than other fats (linoleic and linolenic methyl esters freeze at –35 ºC and –52 ºC, respectively (Knothe and Dunn, 2009). Also, due to the large number of *cis* double bonds in these fatty acids their mobility is better under low temperature conditions. Therefore, the molecules in flax oil were slow to release energy to the environment and resulting in a larger T2. Previous studies showed that longer T2 is provided by highly mobile components, while restricted motion contributes to smaller T2 (Sun & Isayev, 2007).

As temperature is lowered, T2 decreases with increasing Lorentzian width. Below 248 K the peak at 3.7 ppm increased sufficiently to interfere with the peak at 4.2 ppm increasing the difficulty of analysis.

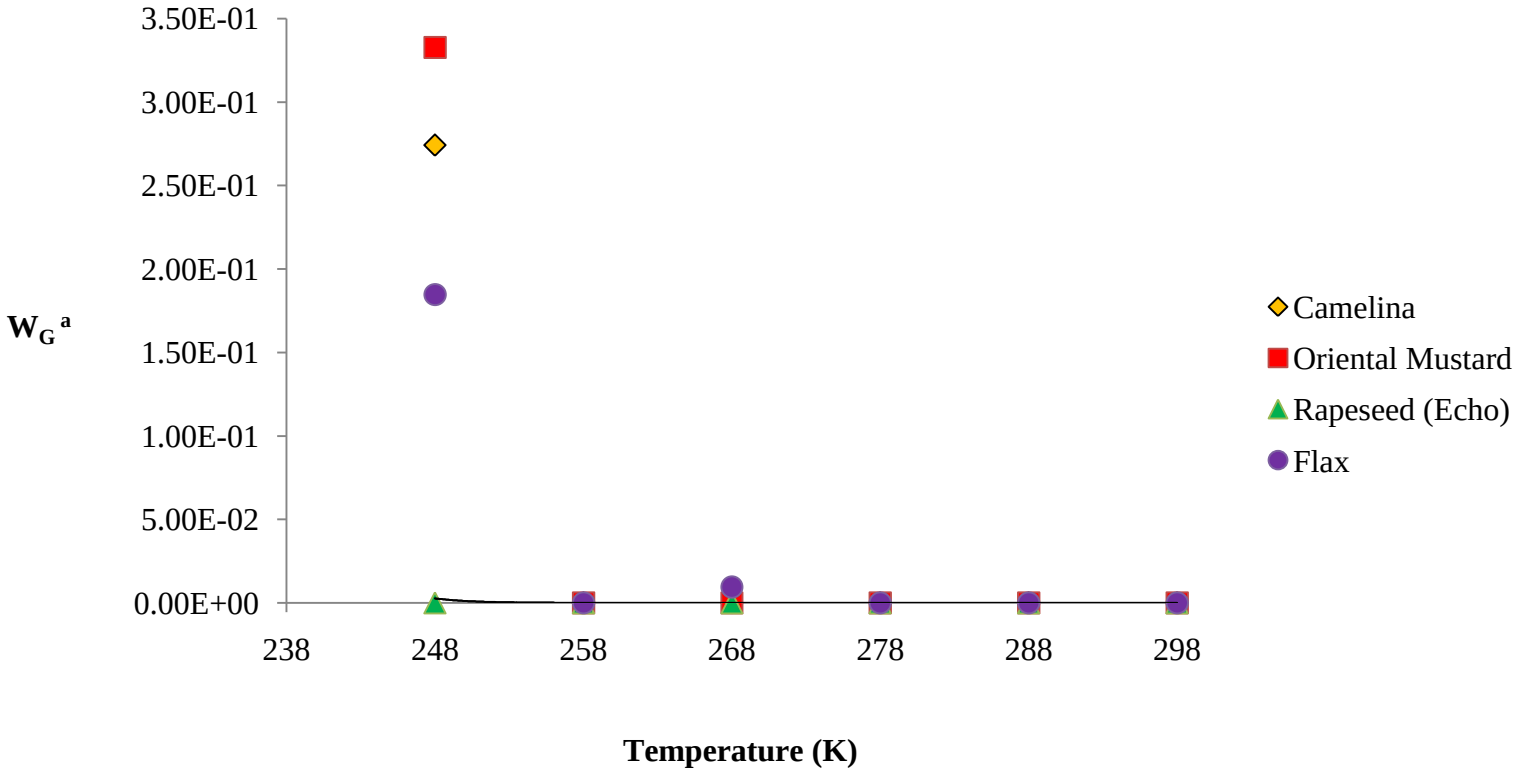


Figure 5.3 The relationship between singlet Gaussian width (4.2 ppm) and temperature for four oils

[a] Gaussian width

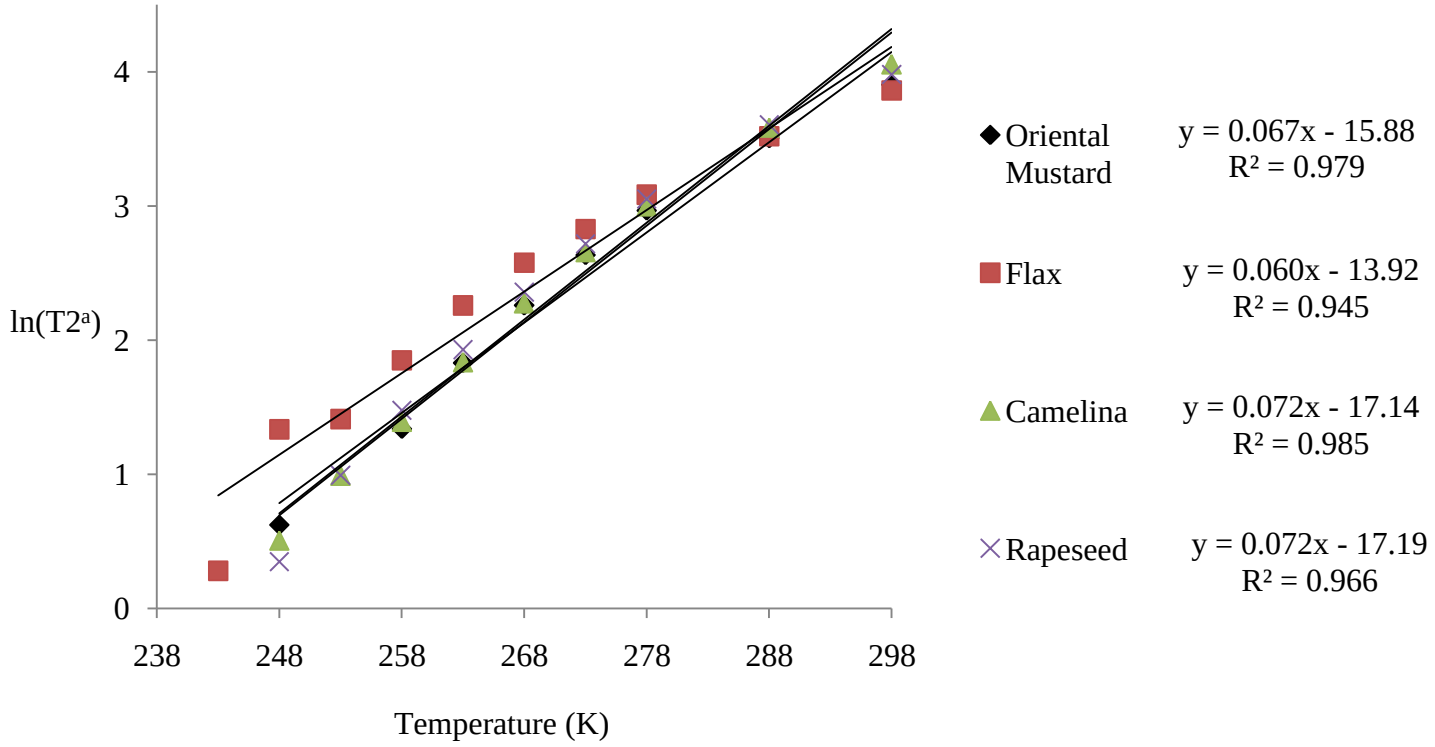


Figure 5.4 Relationship between T2 and temperature for four different vegetable oils
[a] spin-spin relaxation time

5.5 Conclusions

The oxidative stability of vegetable oil esters derived from vegetable oils decreased after filtration through silica or celite columns due to the loss of natural anti-oxidants. In addition the OSI of TMP TE containing excess FAME was likely lower than the OSI of pure TMP TE.

The ^{1}H-NMR proton relaxation was useful in measuring the molecular motion of methylene protons in TMP esters. The T2 was useful in tracking the low temperature environment of various fatty acid esters. The results indicated that the log of the spin-spin relaxation time is linearly correlated with rising temperature. According to the Gaussian widths of the singlet the fluidity of molecules was still substantial above 248 K.

CHAPTER 6
GENERAL DISCUSSION

This thesis reveals a strategy for identifying vegetable oils with low temperature flow properties and good resistance to oxidation. Vegetable oils with these improved properties have potential as a feedstock for production of synthetic ester lubricant base oils. The high correlations observed between saturated fatty acid content and oil physical properties strong linear relationship between the PP of methyl esters and the saturate fat content. Three *B. rapa* L. cultivars were identified as a source of oil with excellent cold fluidity (−21 ^{o}C for ME PP). These oils had a trait of low combined palmitic and stearic acid content. It was also found that oils that are low in PUFA had superior oxidative stability. *B. rapa* cv. Echo single plant selections with less than 20% of combined linoleic and linolenic acids was identified with excellent stability (OSI=12 h). Selections from the same cultivar with higher PUFA content had lower stability. These results reveal that the properties of low temperature flow and oxidative stability might both be further improved. For example, it is suggested that oil from a cultivar with less than 2.59% saturated fat and less than 20% PUFA content would have superior physical and chemical properties for application as a lubricant. This finding provides the guidelines of choosing feedstock for biolubricant production.

Chemical modification of TAG molecules may be required to produce a superior lubricant. Replacing the glycerol backbone by more stable branched polyol called TMP may be necessary for making a suitable base oil for many applications. Base catalyzed transesterification reactions were compared to determine their efficacy in catalytic conversions of FAME and TMP to TMP TE. A new reaction procedure (reverse addition) and lower cost base catalyst (potassium carbonate) successfully converted a high portion of FAME and TMP to the desired esters. Traditional base catalyzed transesterification using sodium methoxide conducted in a one-pot reaction was compared with revered addition reactions catalyzed by less expensive and toxic bases. Utilizing lower cost and safer potassium carbonate, as the catalyst would increase the

efficiency of large-scale reactions while reducing the danger of catalyst use. Other reaction parameters including reaction time and mole ratio of FAME to TMP were also optimized. In the study, TMP was efficiently converted (96.4%) to TMP TE with K_2CO_3 catalyst (1% of w/w K_2CO_3 catalyst, 2 h reaction time, 3.9 mole ratio of FAME to TMP, 130 ^oC reaction temperature and 28 mm Hg).

The next study was designed to characterize the chemical and physical properties (oxidative stability and low temperature flow) of a mixture of TMP TE and FAME. The results indicated a loss of oxidative stability when the TMP TE was synthesized and refined. The lost stability of the oil may be a result of the oil refining process due to the removal of natural anti-oxidants or to exposure of the oil to high temperatures during the transesterification reaction while in contact with strong base. In addition the presence of unreacted FAME with the TMP TE may also have contributed to decreased oil stability. ^{1}H-NMR relaxation analysis of TMP esters derived from four oils revealed the power of this non-destructive analytical tool for identification and characterization of the solution and gel properties. Spin-spin relaxation time (T2) can be used to probe the molecular motion of the protons due to the different chemical environment at different temperatures. The results showed that T2 of a peak with a chemical shift at 4.2 ppm was positively correlated with temperature. ln(T2) increased linearly with temperature.

In the future, it is suggested to further investigate methods to improve the purification process to both remove the unreacted FAME as well as the spent catalyst. The presence of FAME in the crude sample strongly influenced the properties of the TMP TE. Therefore, it is important to obtain pure TMP TE for future studies. Furthermore, reaction design for industrial scale transesterification could be further explored. Some of the practical problems (foaming and handling safety) have been solved in this study. A practical solution for the production of pure TMP TE would be recommended before an industrial scale reaction can be recommended. Lastly, it is also beneficial to further identify an oil source with less than 2.59% saturated fat and less than 20% PUFA content. Once the cultivar was identified it can be used as a continuous supply of biolubricant feedstock with excellent physical properties.

CHAPTER 7
GENERAL CONCLUSIONS

With the growing depletion of petroleum resources and concern over environmental issues associated with utilization of non-renewable resources the potential for using vegetable oils as a low cost and renewable feedstock has gained the attention of scientists. However, natural vegetable oils have poor oxidative stability and cold flow properties compared to products derived from petroleum. It is important to have lubricant with excellent low temperature flow properties for operation in the winter in North America as temperatures may reach −50 ^{o}C. Therefore, the thesis introduced two possible methods to develop a polyol-based biolubricant that has excellent cold flow performance and oxidative stability. In addition, the production cost and possibility of large-scale production were also considered in this project.

At first a feedstock was identified with low temperature flow properties and oxidative stability that exceeds the properties from other sources. The effects of fatty acid profile on biodiesel PP and oil OSI of different oilseed species were explored. The study illustrated that low temperature flow properties and stability are determined by two factors: saturated fat and PUFA content, Oils derived from oilseeds with less than 2.59% saturated fat and 20% PUFA content may provide excellent properties oil for lubricant production. *B. rapa* cv. Echo was found to have the best physical and chemical properties among all oilseeds. The methyl esters PP at −21 ^{o}C and OSI of 12 h were observed in this cultivar. Choosing Echo as the biolubricant feedstock provides a superior fatty acid profile for developing a motor with PP of −50 ^{o}C.

The second method was to improve the oil physical properties by chemical modification of plant TAG molecule structure with a branched polyol called TMP.A two-step base-catalyzed transesterification reaction was applied and optimized using potassium carbonate as a safe and inexpensive catalyst. In the second stage reaction, more than 96% conversion rate was achieved within 2 h and 100% conversion rate was achieved when the reaction proceeded for 18 h. This superior conversion was only possible when using a reverse addition reaction procedure. The

results obtained from the base catalyzed transesterification reaction also showed that the production cost could be reduced by utilizing a lower cost base catalyst (potassium carbonate). Traditionally, base catalyzed transesterification reactions were conducted as one-pot reactions utilizing sodium methoxide. Sodium methoxide is considered expensive and hazardous when used in industrial scale processes. It was shown that the lower cost and safer potassium carbonate catalyst was effective making it a superior choice for large scale of reactions with lessened concerns of danger from the catalyst.

This work showed two possible ways to improve physical properties of vegetable oils. Selection of the best cultivars with excellent properties and chemically modifying the oil structure were proven to effectively improve oil low temperature behavior and oxidative stability. Some further investigation such as separation of FAME and TMP TE as well as identifying a cultivar low in saturated fat and PUFA content would achieve the goal of producing −50 ^{o}C motor oil.

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APPENDIX A
SAMPLE CALCULATION

Molecular Weight (MW) Calculation of Reactants

MW of methanol=32.04 g/mol
MW of TMP=134.17 g/mol
MW of K_2CO_3= 138.205 g/mol
MW of KOH= 56.1056 g/mol
MW of NaOMe= 54.02 g/mol
Average MW of fatty acid =279.6 g/mol
MW of TG=278.6×3+14+13+14=876.8 g/mol
MW of ME= 278.6+15=293.6 g/mol

<u>First step of transesterification</u>

Mass of vegetable oil=100 g
Mole of vegetable oil=100 g/876.8 g/mol=0.114 mol
Mole of methanol=6×mole of vegetable oil=0.114×6=0.684 mol
Mass of methanol=32 g/mol×0.684 mol=21.93 g
Mass of KOH= Mass of vegetable oil ×0.5%=0.5 g

<u>Second step of transesterification</u>

Mass of TMP=10 g
Mole of TMP=10 g/134.17 g/mol=0.075 mol
Mole of FAME=Mole of TMP × 3.9=0.29 mol
Mass of FAME=293.6 g/mol×0.29mol=85.34 g
Mass of K_2CO_3= (85.34 g+10 g) ×1.0%= 0.95 g

APPENDIX B

LOW TEMPERATURE TEST OF ^{1}H-NMR

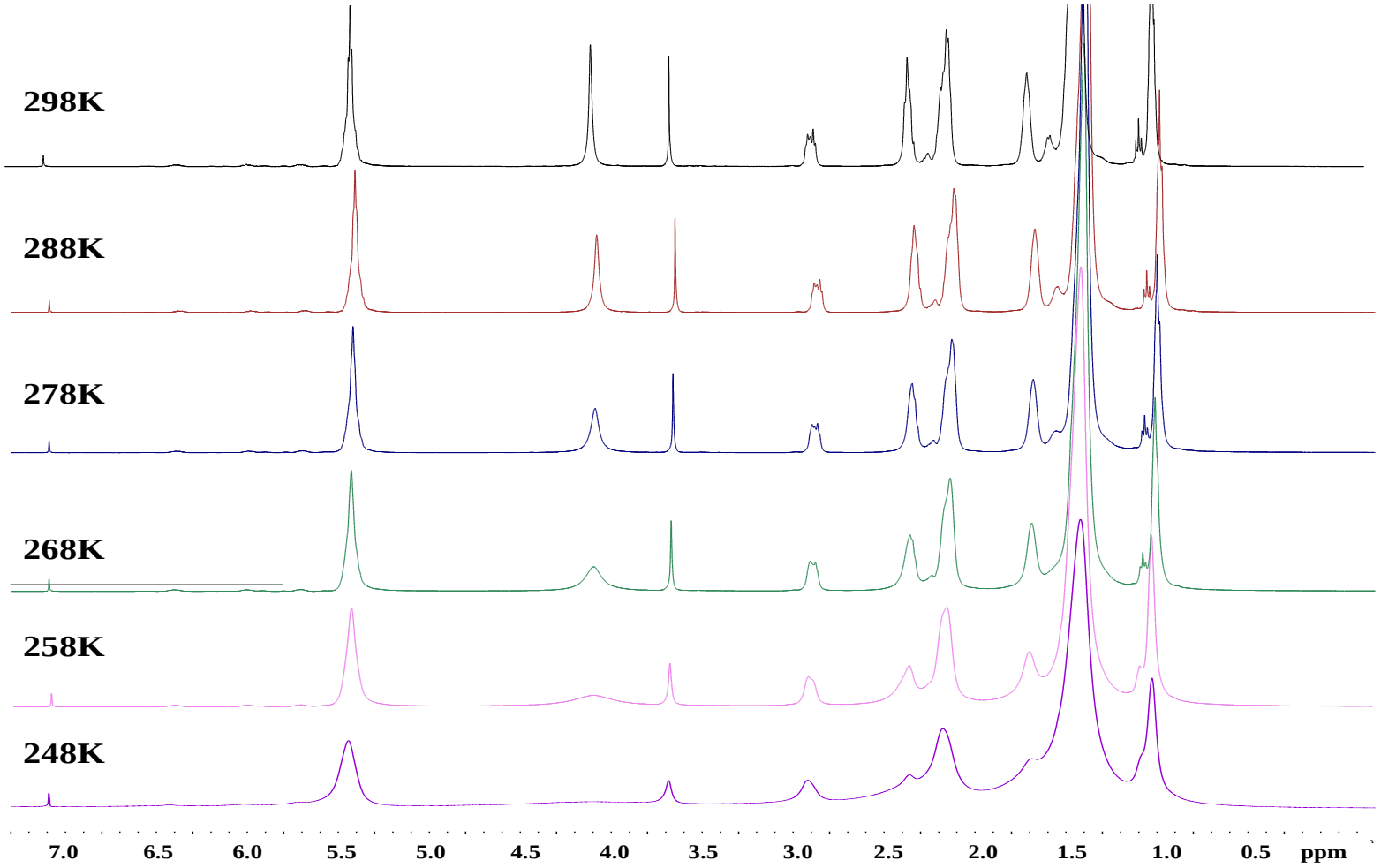

Figure 10.1 Multiple ^{1}H-NMR spectra of oriental mustard TMP TE [a] 298 K to 248 K

[a] trimethylolpropane triesters

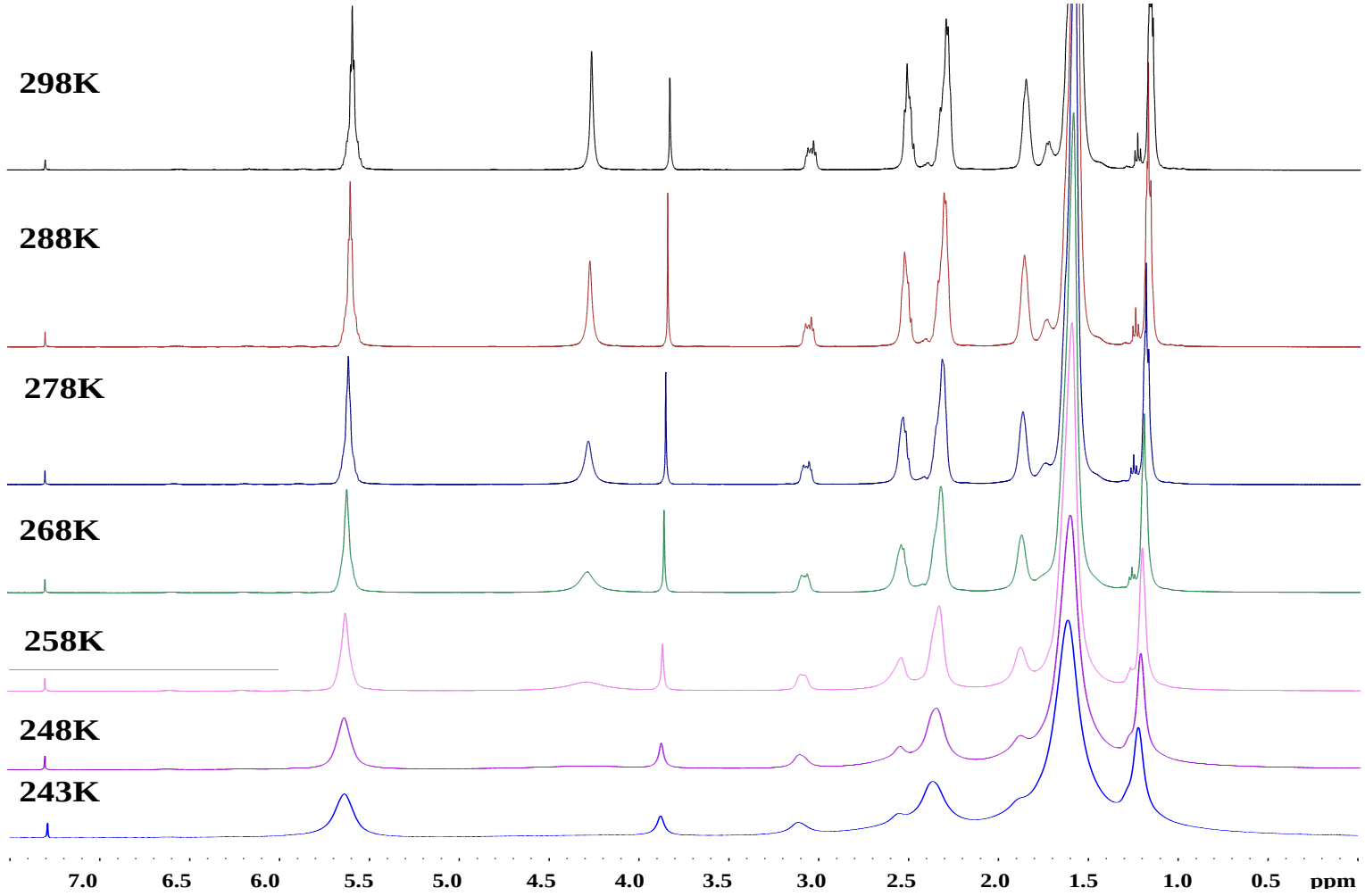

Figure 10.2 Multiple ^{1}H-NMR spectra of rapeseed Echo TMP TE [a] from 298 K to 243 K

[a] trimethylolpropane triesters

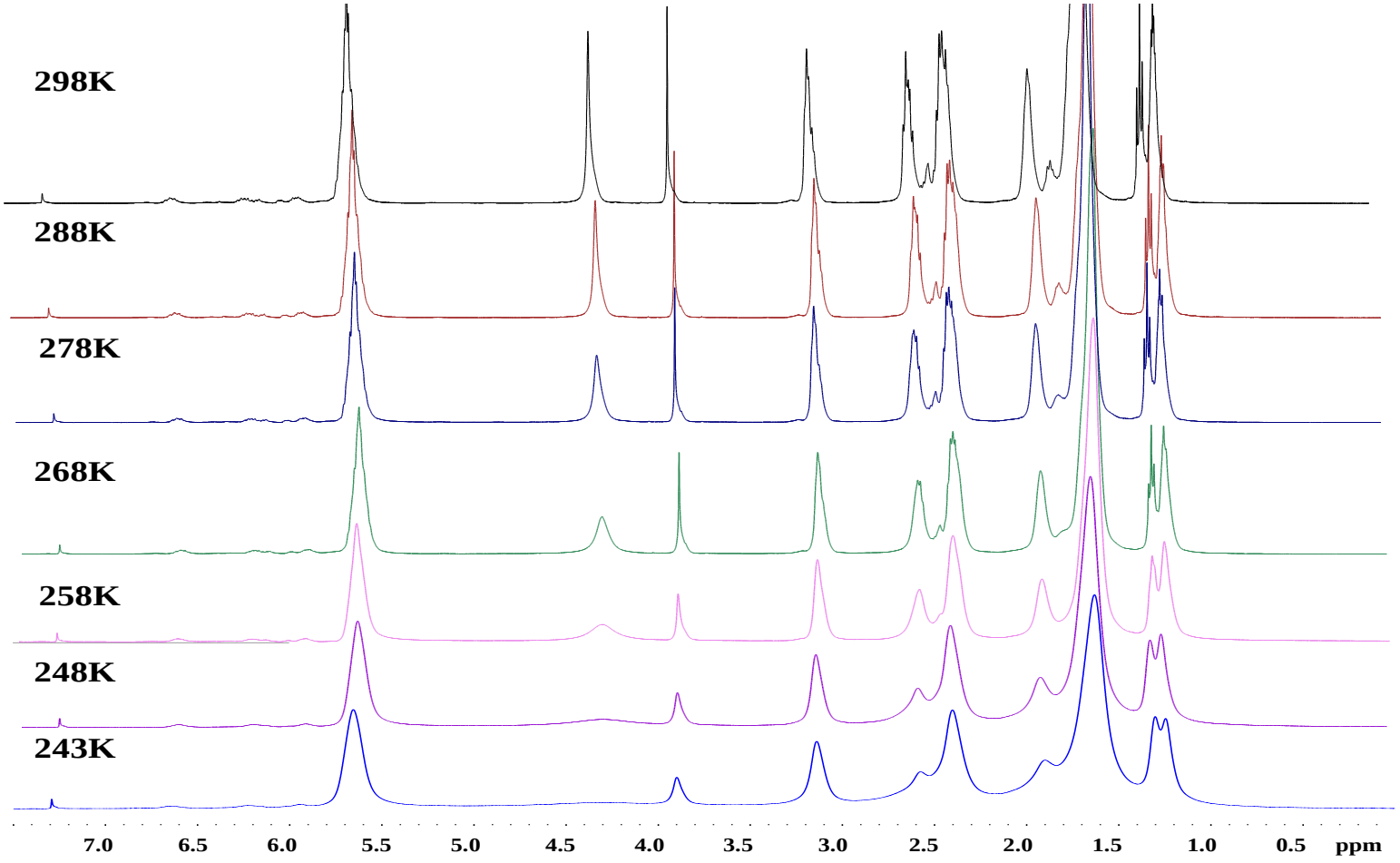

Figure 10.3 Multiple ^{1}H-NMR spectra of flaxseed TMP TE [a] from 298 K to 243 K

[a] trimethylolpropane triesters

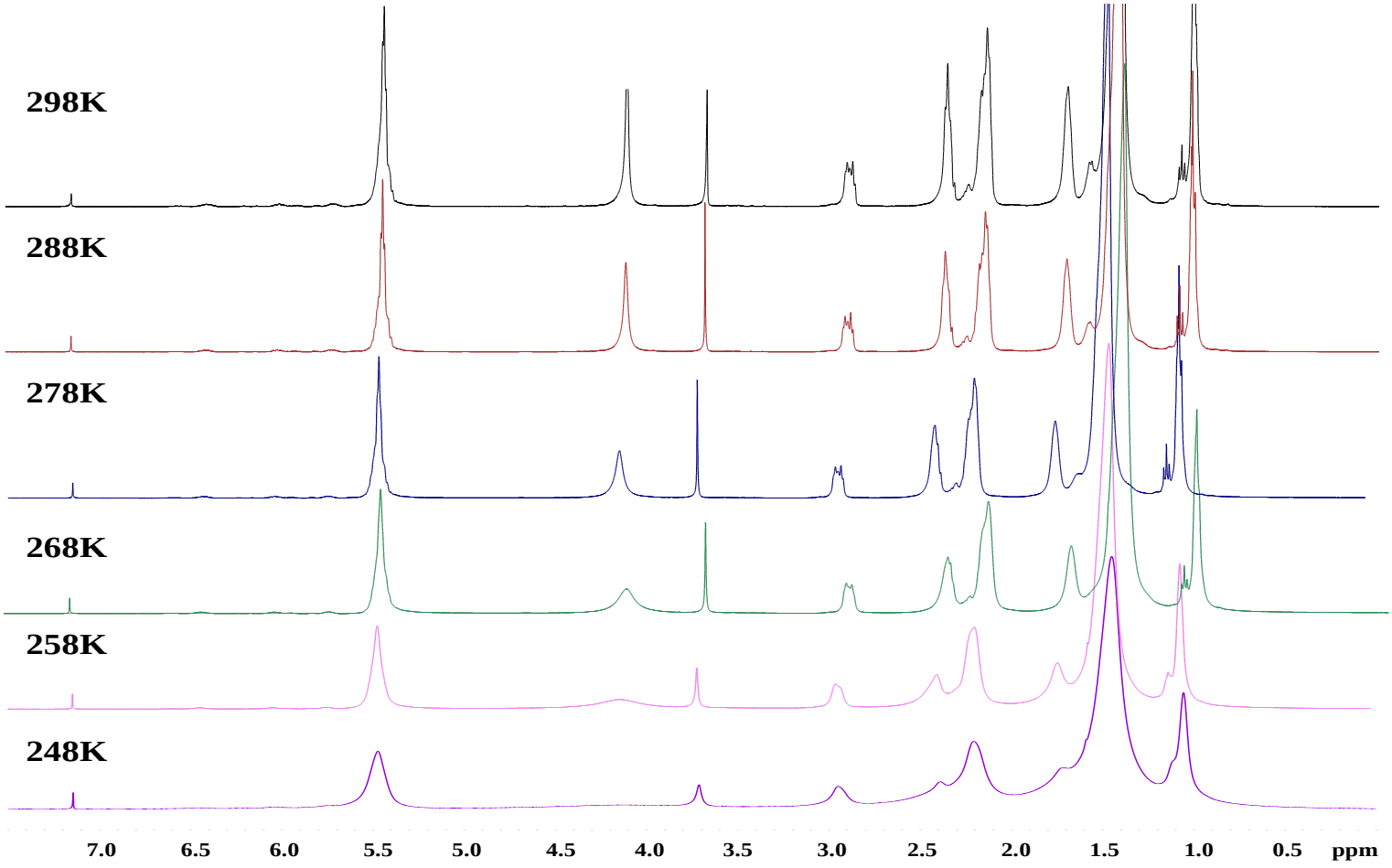

Figure 10.4 Multiple ^{1}H-NMR spectra of camelina TMP TE [a] from 298 K to 248 K
[a] trimethylolpropane triesters