Measurement and Separation of Sterol Glycosides in Biodiesel and FAME

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Abstract

The major issue that hinder the widespread use of biodiesel is its poor cold weather stability and operability. This is attributed minor components identified as monoglycerides (MG), diglycerides (DG) and sterol glycosides (SG). There is currently no standard method to determine SG levels in biodiesel.

A method to isolate and measure SG concentration in biodiesel and FAME was first developed. This was accomplished by decompatibilizing SG from the biodiesel matrix using n-dodecane and purifying the solids using a Folch liquid-liquid extraction. The extracted SG was analyzed by GC-FID; the tricaprin internal standard was detected at 21.5 min and SG from 26-26 min. Recovery using this method was 100% ± 2.5% when 3 commercial canola biodiesel samples were spiked with 38 ppm SG and extracted.

This method was used to measure SG concentration of filtered FAME produced using 0.3wt%, 0.5wt% and 0.7wt% at a MeOH:Oil (mol/mol) ratio of 4:1, 5:1, 6:1, 7:1 and 9:1. The biodiesel produced was characterized according to ASTM D6584; MG, DG and TG decreased with increasing catalyst concentration and MeOH:Oil ratios. The SG solubility in reactive FAME was found to be lowest at high glycerol catalyst concentration. High levels of TG were found to solubilize SG in the reactive FAME. Finally, the solubility of SG in reactive FAME increased when high ratios of methanol were used.

Résumé

Le problème majeur qui empêche l'utilisation du biodiesel est sa mauvaise stabilité et opérabilité à basse température. Ceci est attribué aux composants mineurs présents, identifiés comme des monoglycérides (MG), des diglycérides (DG) et les glycosides de sterol (SG). Actuellement, il n'y a pas de méthode standard pour déterminer la concentration de SG dans le biodiesel.

Une méthode pour isoler et mesurer la concentration de SG dans le biodiesel et FAME a d'abord été développée. Ceci a été accompli par la decompatibilization du SG en utilisant du n-dodécane a basse température, et en utilisant une extraction liquide-liquide Folch pour isoler les composants non-polaire. Le SG isolé a été analysé par GC-FID; le standard interne a été détectée à 21,5 min et le SG de 26 à 26 min. La récupération en utilisant cette méthode était de 100% ± 2,5% lorsque 3 échantillons de biodiesel de canola commerciale ont été dopés avec 38 ppm SG et analysé.

Cette méthode a été utilisée pour déterminer la concentration de SG dans du FAME filtrée produite en utilisant 0,3%cat, 0,5% cat et 0,7% cat à un ratio MeOH: Huile (mole / mole) de 4: 1, 5: 1, 6: 1, 7: 1 et 9: 1. Le biodiesel produit a été caractérisé selon la norme ASTM D6584; le MG, DG et TG ont diminués avec la concentration de catalyseur croissante. La solubilité du SG dans la phase de FAME réactif a été jugée plus faible quand la concentration de catalyseur dans le glycérol était élevée. Des niveaux élevés de TG ont solubilisés le SG dans la phase de FAME réactive. Enfin, la solubilité du SG dans le FAME à augmenter lorsque des niveaux élevés de methanol ont été utilisés.

Statement of Contributions and Collaborators

I hereby declare that I am the sole author of this thesis. I performed all the experiments and the data analysis. I have written all chapters and produced all visual material in this thesis.

Dr. Andre Y. Tremblay supervised this thesis project and provided continual guidance and support. He also helped with editorial comments and corrections to the written work presented. His day-to-day guidance, discussion and support have resulted in a tremendous improvement in this thesis that would not have been possible otherwise.

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Nomenclature

This section lists some of the most frequently used abbreviations. It is by no means exhaustive, but is meant to guide the reader through the bulk of the material. Other, less-common, abbreviations are specified at the first instance.

ASG – Acylated Sterol Glycosides

ASTM – American Society for Testing and Materials

ATR-FTIR – Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy

Bx – Volume fraction of biodiesel in diesel fuel (e.g..: B20 = 20vol% biodiesel, 80vol% diesel)

CAT (wt%) – Catalyst (sodium methoxide) used, weight percent on an oil used basis

DG – Diglycerides

EN – European Norm

FAAE – Fatty Acid Ethyl Ester

FAME – Fatty Acid Methyl Ester

GC-FID – Gas Chromatography – Flame Ionization Detector

MeOH - Methanol

MG – Monoglycerides

MSTFA – N-Methyl-N-(trimethylsilyl)-TrifluoAcetamide

MWCO kDa – Molecular Weight Cut-Off, measured in kilo Daltons (10^3 g/mol)

NPD – Net Boiling Point

SPE - Solid Phase Extraction

SG – Sterol Glycosides

TC - Tricaprin

TG – Triglycerides

Chapter 1

Introduction

1. Introduction

Biodiesel is an important category of renewable fuels as it permits the use of existing fuel transportation infrastructure and current diesel engine technology with little to no modifications [1]. Biodiesel is a renewable and biodegradable fuel made from vegetable oils, animal fats or algae oil via the transesterification of triglycerides in alcohol. Raw materials used in the production of biodiesel can often be sourced. Currently, most biodiesel is made using methanol producing Fatty Acid Methyl Ester (FAME) and from ethanol producing Fatty Acid Ethyl Ester (FAEE). Industrial methanol is made from natural gas, a non-renewable source. The amount of non-renewable carbon added to the FAME molecule is very small (typically 1/16 to 1/18 of the total carbon in a FAME molecule). There are ways to produce methanol from methane in biomass and ethanol from the fermentation of sugars, both of which are renewable sources.

The main barrier-to-entry into the broader use of this fuel is its poor cold weather properties and limited storage life. The presence of impurities, such as partially reacted glycerides and sterol glycosides (SG), is reported to be the main reason behind the high operating temperature of biodiesel.

1.2 Objectives

There are two major objectives in this thesis. The first objective is the development of a new quantitative method to determine SG concentration in biodiesel and FAME using readily available analysis techniques such as GC-FID. The second objective of this thesis was to study the solubility of SG in a reactive FAME mixture by filtration through a ceramic membrane using the

new method to measure SG concentrations in both the retentate and permeate. The separation factor was then calculated and the solubility of SG in the reactive FAME phase.

1.3 Thesis structure

Chapter 2 will present an overview of the history of biodiesel, the chemistry and production of the fuel and the properties of biofuels and conventional petroleum fuels.

Chapter 3 will cover a review of the transesterification reaction, production processes, biodiesel contaminants and their analysis.

Chapter 4 describes the development of a novel technique to extract and measure sterol glycoside content in biodiesel and FAME using equipment and ASTM techniques typically used in biodiesel testing facilities.

Chapter 5 describes the use of this analytical technique to study the solubility of sterol glycosides in a reactive FAME mixture through the use of a ceramic membrane.

Finally, chapter 6 will cover conclusions, and recommendations for future work.

Chapter 2

Overview

2. Overview

2.1 Definition

Biodiesel is a mixture of fatty acid esters obtained from the transesterification of vegetable oil or animal fats. The criteria in ASTM D6584 and EN14214 outline a limit on the amount of glycerol, partially reacted glycerides and free water allowed in a sample of fuel. The nomenclature of biodiesel is based on the type of alcohol used in the reaction (Fatty Acid Methyl Ester, FAME and Fatty Acid Ethyl Ester, FAEE). Biodiesel and conventional diesel fuel share many physical characteristics; it can be used directly in its neat form (B100) or blended with conventional diesel in different proportions [2].

2.2 History of biofuels.

The transesterification of vegetable oil was first performed as early as 1853 by Patrick Duffy, 40 years before the introduction of the diesel engine by Rudolf Diesel. On August 10th 1893, a model of Diesel's engine ran on peanut oil, marking August 10th as International Biodiesel Day. During the 20's and 30's, and later during WWII, many countries including Germany, France, England and Japan were reported to have used vegetable oils in place of conventional diesel. Due to the high viscosity of vegetable oils, these experiments led to clogged fuel injectors and engine coking. On August 31st 1937, G. Chavanne (University of Brussels, Belgium) was awarded a patent for the transesterification of vegetable oils "Procédé de Transformation d'Huiles Végétales en Vue de Leur Utilisation comme Carburants". This patent describes the transesterification of vegetable oils using ethanol and is the first account of production of what is now known as biodiesel [1].

Due to the widespread availability and low cost of petroleum diesel fuel, biodiesel has gained little traction, except in times of high petroleum prices and shortages. The current impact of fossil

fuels on climate change has increased the interest in renewable fuels. Since the carbon found in biodiesel originates from the atmosphere, biodiesel is said to be less carbon intensive than conventional petroleum products [3] [4]. In addition to this, biodiesel produces considerably less particulate matter emissions compared to conventional diesel fuel. Because biodiesel contains molecular oxygen, the nitrogen oxide (NO_X) emissions are greater than in conventional diesel. This can be reduced by hydrogenating the ester and removing the oxygen in the fuel [5] or by using urea-SRC (selective catalytic reduction) to transform NOx into N_2 which greatly reduces GHG emissions.

2.3 Diesel Engine

The diesel engine is used in a wide range of heavy duty applications such as ground and maritime transportation and the agricultural industry due to its high fuel economy, high torque and power relative to a gasoline engine. The absence of electronic components such as spark plugs increases its reliability. As seen in Figure 2-1, diesel fuel accounts for about 22% of petroleum products used in 2011.

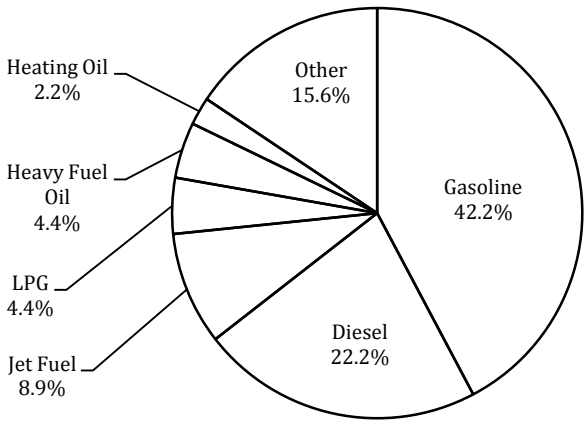

Figure 2-1: Products made from petroleum **[6]**

Since biodiesel can be used in an unmodified diesel engine, it is a fast growing alternative to petroleum-derived diesel fuel due to the low transition costs.

2.4 Diesel combustion

Diesel combustion differs from gasoline combustion as it undergoes spontaneous combustion due to high compression ratios (20:1) and does not rely on combustion through ignition like a gasoline engines. Air and finely atomized diesel fuel are injected in the cylinder which compresses the mixture until the diesel fuel spontaneously ignites. The power of the engine is controlled by the amount of fuel injected during each power cycle, eliminating the need for a throttle [7].

2.5 Diesel fuel

Diesel fuel (ASTM D975) is a C_{10}-C_{15} mixture characterized by its boiling point range of 180°C-360°C vs a range of 80°C-200°C for gasoline (ASTM D4814). Diesel fuel can be found in grades No. 1 and No. 2 which have different chemical compositions. Diesel No.1 has better cold flow properties and is generally used in winter. Their compositions are similar, as seen in Table 2-1 [8]. Diesel stations often stock both grades and blend them on-site according to weather conditions and demand.

Table 2-1: Hydrocarbons in diesel fuel oil No. 1 and No. 2

Hydrocarbon type	Volume % Fuel oil No.1	Fuel oil No.2
Paraffins (n- and iso-)	52.4	41.3
Monocycloparaffins	21.3	22.1
Bicycloparaffins	5.1	9.6
Trycycloparaffins	0.8	2.3
Total Saturated Hydrocarbons	79.7	75.3
Olefins	-	-
Alkylbenzenes	13.5	5.9
Indans/tetralins	3.3	4.1
Dinaphthenobenzes	0.9	1.8
Naphthalenes	2.8	8.2
Biphenyl/acenaphthalynes	0.4	2.3
Fluorenes	-	1.4
Phenanthrenes	-	0.7
Total Aromatic Hydrocarbons	23.6	24.7

2.6 Fuel characteristics

2.6.1 Soot formation

Soot formation in a diesel engine is dependent on the combustion of the fuel in the engine. The power in a diesel engine is controlled by the rate of fuel injected and not fuel-air ratio. A diesel engine will provide enough oxygen for complete combustion when idle, but not when more power is needed. This leads to the formation of partially combusted particulate matter, or soot. Diesel soot is mostly composed of polyaromatic hydrocarbons (PAHs), known carcinogens, produced from the combustion of aromatic compounds found in the fuel. Biodiesel made from vegetable oils or animal fats does not contain aromatic hydrocarbons and does lead to the formation of PAHs [7]. Additionally, biodiesel contains molecularly bound oxygen which helps complete combustion, further reducing the risk of soot formation and particulate matter.

2.6.2 Cetane Number

The cetane number is the indication of the combustion speed of a substance. It is an inverse function of the ignition delay and indicates the time between the start of the injection and the start of combustion. Higher cetane number fuels will have a shorter delay, resulting in less unburned fuel [9]. As seen in Table 2-2, biodiesel has a higher cetane number than conventional diesel fuel.

Table 2-2: Cetane number of various liquid fuels **[10]**

Fuel	Cetane Number
Diesel	44
Ethanol	8
Methanol	5
B20	46
B100	54
Vegetable oil	45

2.6.3 Emissions

Under ideal conditions, conventional diesel fuel undergoes complete combustion and only produces CO_2 and H_2O. The presence of nitrogen and sulphur can produce NO_X and SO_X which interact with water vapour in the atmosphere and contributes to acid rain formation. The U.S Environmental Protection Agency has limited the sulphur content in diesel fuel to 500ppm [11]. Biodiesel made from vegetable oils contains very few sulphurous compounds, but animal fats can contain high levels and need to be pre-treated in order to satisfy EPA sulphur requirements.

2.6.4 Viscosity

Neat vegetable oil has a higher cetane number than conventional diesel. The reason straight vegetable oil cannot be used in place of diesel is the difference in viscosity. A higher viscosity is not desired because it can affect atomization of the fuel and lead to incomplete combustion resulting in engine coking and carbon deposits [1].

2.6.5 Low Temperature Operability

The low temperature operability characterizes the fuel's behaviour at low temperature. A fuel's low temperature operability is influenced by the feedstock composition and the conversion of the transesterification reaction. Table 2-3 below shows the ester profile of different vegetable oil-based biodiesels and its effect on cold flow properties.

Table 2-3: Cold flow properties vs saturated ester content of different biodiesels **[12]**

Better Cold Flow Properties ↑

	Saturated [%]	Monounsaturated [%]	Polyunsaturated [%]
Canola	8	60	32
Safflower	10	15	75
Sunflower	12	16	72
Corn	13	27	60
Olive	15	10	75
Soybean	15	25	60
Peanut	20	50	30
Cotton Seed	25	20	55
Yellow Grease	35	50	15
Lard	43	47	10
Beef Tallow	48	48	4
Palm	50	40	10
Coconut	90	2	8

Biodiesel made from feedstock with lower saturated triglyceride content have better cold flow properties than biodiesel made from feedstock which contain high levels of saturated oils. Saturated esters tend to have a higher melting point than mono or polyunsaturated esters, and crystalize out of solution at higher temperatures.

The cold flow properties are also influenced by the total conversion of the transesterification reaction. Partially reacted glycerides, such as monoglycerides (MG) and diglycerides (DG), have a

melting point higher than the fuel working temperature. Biodiesel which contains high amounts of partially reacted glycerides will have poor cold flow properties.

The Cloud Point is defined as the temperature at which the waxes in the fuel begin to visibly crystalize. The Pour Point is defined as the temperature at which diesel or biodiesel no longer able to pour freely. ASTM D2500 and D97 are used to determine cloud point and pour point, respectively. The cloud point and pour point of different biodiesels can be found in Table 2-4 below.

Table 2-4: Cloud Points and Pour Points of different biodiesels **[13]**

	Cloud Point [°C]	Pour Point [°C]
Soybean	1	0
Canola	0	-9
Palm	17	15
Jatropha	8	6
Tallow	15	6

2.7 Summary

Although biodiesel has many advantages such as lower emissions and engine compatibility, it has disadvantages that hinder its adoptions as a true alternative to conventional diesel. Low temperature operability and stability are the main issues that need to be addressed in order for biodiesel to become a truly viable alternative fuel.

Chapter 3

Biodiesel Production and Transesterification Mechanism

3. Transesterification Mechanism and Biodiesel Production

Vegetable oil cannot be directly used in diesel engines due to its high viscosity. This can lead to incomplete atomization by the fuel injector and incomplete combustion. There are four common ways to reduce the viscosity of vegetable oils:

1. Blending with conventional diesel
2. Pyrolysis
3. Emulsification
4. Transesterification

Transesterification is widely used and the only method that produces what we know as fatty acid esters [7]. This reaction consists of a triglyceride molecule reacting with 3 molecules of alcohol, in the presence of a catalyst, to produce glycerol and fatty acid esters. The reaction can be found in Figure 3-1.

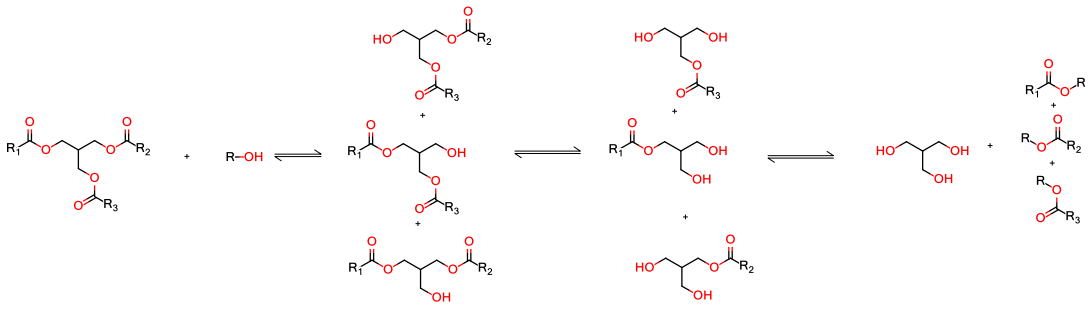

Figure 3-1: Transesterification reaction

The transesterification reaction does not occur in a single step, but rather as a sequence. One ester is stripped at a time resulting in intermediate compounds being produced. These by-products, MG and DG, are partially soluble in FAME. They are difficult to separate from the final product and affect the fuel's quality.

3.1 Feed stocks

Roughly 50% of biodiesel produced in North America is made from soybean oil [14]. The high price of Virgin and Refined, Bleached and Deodorized (RBD) oils makes it difficult for biodiesel production plants to use high-quality feedstock and be economically viable without government subsidies [2]. Lower quality oils, such as yellow grease and waste frying oils, can be used to lower the material costs of producing biodiesel [15]. These waste oils are high in free fatty acids (FFA) which result in the formation of soaps when base catalysts are used. FFA's can be converted to esters using an acid catalyzed transesterification reaction before the base catalyzed reaction. This increases production costs and reduces the advantage of using low quality oils as feedstock [16].

3.2 Alcohols and Catalysts

3.2.1 Alcohols

Methanol is mostly used in the transesterification in North America for is as it is the cheapest alcohol available. In countries where ethanol is cheaper, such as Brazil, fatty acid ethyl ester (FAEE) is produced instead of FAME [7]. The choice of alcohol used in the transesterification reaction can influence the kinetics and yield of the reaction, as seen in Table 3-1 below.

Table 3-1: Kinetics and yield vs different alcohols **[16]**

Alcohol	Yield [%] after 45mins
Methanol	100
Ethanol	98
1-Propanol	90
1-Butanol	80
1-Octanol	65

The presence of water during the transesterification reaction leads to the formation of soap and increases transformation costs. Methanol has the advantage of not forming an azeotrope with water, as seen in Table 3-2. This facilitates alcohol recycling in biodiesel operations.

Table 3-2: Azeotropes of different alcohols/water **[17]**

	Molecular formula	Azeotropic composition mol/mol
Methanol	CH_3OH	1 (no azeotrope)
Ethanol	C_2H_5OH	0.8966
1-propanol	C_3H_7OH	0.4235
1-butanol	C_4H_9OH	0.252
1-pentanol	$C_5H_{11}OH$	0.1429

3.3.2 Catalyst

Catalysts are compounds that lower the activation energy of a reaction without undergoing permanent chemical change. They can be homogeneous or heterogeneous. While heterogeneous catalysts are easier to separate, they tend to be more expensive and produce lower yields of ester compared to homogeneous catalysts. Homogeneous alkali catalysts are typically used because of their mild reaction conditions and high yields. The most common alkali catalysts used in

transesterification are sodium hydroxide (NaOH), sodium methoxide ($NaOCH_3$), potassium hydroxide (KOH) and potassium methoxide ($KOCH_3$) [7].

The major advantage of using methoxides, although more expensive, is that they are virtually water-free and will result in lower soap formation. Hydroxides release water when mixed with alcohols, as seen in Figure 3-2, which will lead to soap formation.

Figure 3-2: Reaction of sodium hydroxide and methanol

3.3 Biodiesel Production Process

The transesterification reaction is carried out under constant agitation as oil and methanol are immiscible [18]. The catalyst, in the form of methoxide ion, is added to the alcohol and the reaction carried out at atmospheric pressure and a temperature slightly lower than the boiling point of the alcohol. The residence time is usually 60 min, after which the reactive glycerol phase is separated from the ester phase by gravity settling. Amphiphilic compounds such as MG, DG and soaps can be suspended in both phases. The catalyst is found mostly in the polar glycerol phase, and is separated out during phase separation.

The ester phase is washed with a weak aqueous acid solution, such as phosphoric acid in water. This removes polar impurities such as glycerol and neutralizes any catalyst present in the phase. The waste water is then distilled to recover unreacted alcohol and the washed ester phase is dried to remove water [19].

3.4 Biodiesel Contaminants

Impurities in biodiesel can affect the fuel's cold flow properties and storage stability. Leftover catalyst and methanol can damage and corrode fuel lines and fuel pumps. Monoglycerides and sterol glycosides (SG) can precipitate and clog fuel filters [20] due to their high melting points.

Biodiesel is hydrophobic and can be separated from hydrophilic and amphiphilic compounds by a water wash. This step allows for the removal of excess catalyst, soaps and glycerol [19]. The water is separated by gravity and the fuel is later dried to remove water droplets.

Other oil-soluble contaminates originate from the initial feedstock oil. Crude soybean oil is a complex mixture of triglycerides and other oil-soluble compounds, as seen in Table 3-3.

Table 3-3: Typical composition of crude soybean oil **[21]**

Component	Sub-component	% [wt]
Triacylglycerol	-	94.4
Phospholipids	-	3.7
Unsaponifiables	Includes SG	1.3-1.6
Sterols	-	0.236
	Campesterol	0.059
	Stigmasterol	0.054
	β-sitosterol	0.123
	Δ5-Avenasterol	0.005
	Δ7-Stigmasterol	0.005
	Δ7-Avenasterol	0.002
Tocopherols	-	0.123
	Alpha	0.0093
	Beta	0.0018
	Gamma	0.0834
	Delta	0.029
Carotenoids	-	0.00008
Hydrocarbons	-	0.38
FFA	-	0.3-0.7
Trace Metals	-	ppm
	Iron	1-3
	Copper	0.03-0.05

Many of these compounds, like tocopherols (Vitamin E), remain soluble in biodiesel after the transesterification reaction. In addition, tocopherols and free sterols exhibit anti-oxidant properties which have a positive effect on fuel storage life.

A class of contaminants that has been recently shown to cause fuel hazing and filterability issues is SG, which is considered unsaponifiable. The acylated form of SG, known as acylated sterol glycosides (ASG), is found in plant and animal cells and is part of the phospholipid layers forming membrane walls within the cell. ASG has been shown to be present from 1 to 125mg/100g fresh weight in over 48 plant sources. It was also shown that the acylated form was 2 to 10 times more abundant than the deacylated form [22]. ASG is very soluble in oil, but the degumming step in the oil refining process can decrease their concentration. During the transesterification reaction, the acylated chain of ASG is removed producing SG. This is insoluble in biodiesel and crystalizes out of solution, creating fuel haze. Unlike MG or DG, SG cannot be reheated and re-dissolved because of its high melting point ($240^{o}C$). The presence of SG at levels as low as 30 ppm [23] may cause the formation of fuel haze, even at room temperature. SG differs from free-sterol and tocopherol, which have been shown to have a positive effect on storage life, due to their antioxidant properties. If SG is present in high enough concentration, it can crystalize and settle at the bottom of storage containers. Cold temperatures can make SG act as a crystal seed for other contaminants, such as MG and DG [24].

In the written literature, there are few references to SG levels in oil and fats, and almost no information exists on its presence in finished biodiesel. Crude soybean oil has been shown to contain 2300 ppm SG, while crude corn and safflower oil contain 500 ppm and 300 ppm respectively [25].

3.5 Gas Chromatography Analysis

Gas chromatography is used to characterize biodiesel and determine the nature and quantity of impurities in the ester [26]. The driving force of gas chromatography is the difference in boiling points of different compounds [1]. Some impurities found in biodiesel have boiling points much higher than the GC working temperature. It is therefore necessary to transform these compounds in order to render them more volatile. This is done by silylating the free hydroxyl groups of the sample using N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) prior to analysis, as seen in the reaction in Figure 3-3. It is important to dry the biodiesel sample to remove any water or methanol which would quench the sylilation reaction.

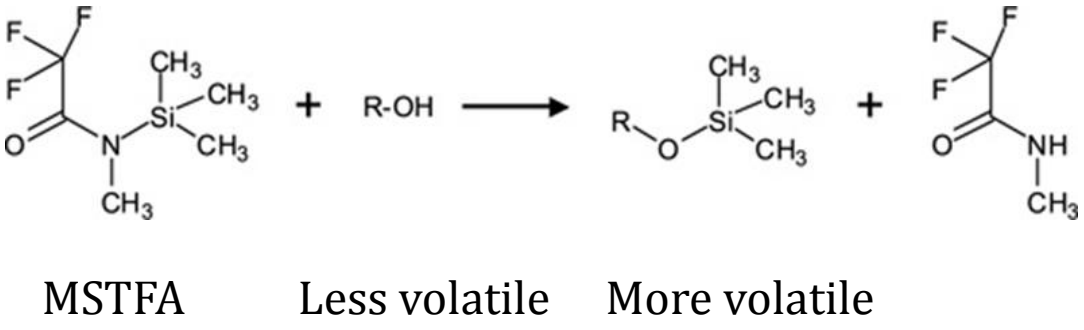

Figure 3-3: Sylilation reaction Using MSTFA

The column parameters and calibration curve procedures were based on ASTM D6584: "Determination of Total Monoglycerides, Total Diglycerides, Total Triglycerides, and Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography". A deviation from this ASTM method was the omission of one of the internal standards, 1,2,4-butanetriol, which is used to determine the composition of free glycerol. A list of standards used in the ASTM analysis of biodiesel can be found in Table 3-3.

Table 3-4: Standards used in GC analysis (Based on ASTM D6584)

Standard (CAS #)	Concentration (µg/mL pyridine)
Glycerol (56-81-5)	500
Monoolein (MG) (111-03-5)	5000
Diolein (DG) (2465-32-9)	5000
Triolein (TG) (122-32-7)	5000
1,2,4-butanetriol (42890-76-6)	1000
Tricaprin (621-71-6)	8000
Sterol Glycosides (Not included in ASTM D6584)	5000

The areas of the peaks of interest were measured and the mass of each component determined by the method outlined in ASTM D6584.

The GC used in this manuscript is a Varian CP-3800 with an auto-sampler attachment. The column used was a 15 m x 0.32 mm x 0.1 µm Restek (MXT – Biodiesel TG) column with a 2 m x 0.53 mm guard column. Calibration curves were made using tricaprin as the internal standard and four references (monoolein, diolein, triolein and soybean SG) standards were used to determine minor components. The operating conditions from ASTM D6584 can be found in Table 3-5.

Table 3-5 : GC operating conditions (ASTM D6584)

Injector		
Cool on column injection		
Sample size	1 µL	
Column Temperature Program		
Initial temperature	50°C	hold 1 min
Rate 1	15°C / min to 180°C	
Rate 2	7°C / min to 230°C	
Rate 3	30°C / min 380°C	hold 10 min
Defector		
Type	Flame ionization	
Temperature	380°C	
Carrier Gas		
Type	Hydrogen or helium	measured at 50°C
Flow rate	3 mL/min	

Chapter 4

A Quantitative Method of Analysis for Sterol Glycosides in Biodiesel and FAME using GC-FID

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4. A Quantitative Method of Analysis for Sterol Glycosides in Biodiesel and FAME using GC-FID

Abstract

Sterol glycosides (SG) are known to cause filter blocking problems in biodiesel use. The extraction and quantitative analysis of SGs is difficult due to its low problematic concentration and interactions with compounds present in biodiesel. The purpose of this study is to develop a method to quantify SG in FAME and biodiesel using gas chromatography and other equipment found in laboratories performing biodiesel analyses. SG was isolated from FAME by decompatibilization using n-dodecane. The solids were further separated by phase partition with a Folch wash, followed by a final n-dodecane rinse. This solution was analyzed by GC-FID using the operating conditions outlined in ASTM D6584. A calibration curve was produced and a first order polynomial was found to offer a good fit ($R^2 = 0.99$).

The lower detection limit of SG using this method was found to be 1 ppm in FAME based on a workable analysis method that is not expected to overload the GC column. Reproducibility tests were performed on soybean and canola biodiesel samples spiked with SG. The recovery of SG by the new method was found to be 99% for soy with a standard deviation of 0.7 % and 100% with a deviation of 3.5 % for canola. The low detection limit and high reproducibility of the new analytical method could form the basis of an ASTM standard for SG analysis in biodiesel.

Key Words: Sterol Glycosides, SG, GC-FID, FTIR, Biodiesel, FAME, Soybean Oil

4.1 Introduction

Biodiesel is a renewable and environmentally friendlier alternative fuel to conventional fossil fuel diesel. Minor components in biodiesel, such as water, free and partially bonded glycerides, free fatty acids and unsaponifiable matter, can affect its performances and quality.

Plant sterols are an important class of organic molecule that occur naturally in cell walls and play a role in the membrane structure. They are found in many forms such as free sterols, or linked to a glucose molecule (SG) which can be esterified (acylated sterol glycosides). SG has been found to have a major impact on the cold weather operability of biodiesel. During the transesterification reaction, the acylated form (ASG) is converted to SG by alkali deacylation, as seen in Figure 4-1 below. The conversion of ASG to SG during the transesterification reaction results in a higher sterol glycoside concentration in the finished biodiesel.

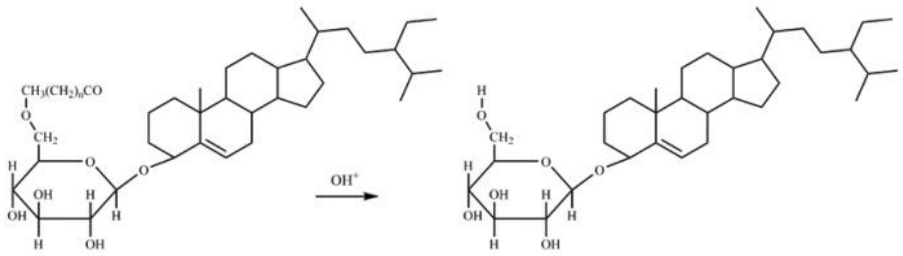


Figure 4-1: Deacylation of acylated sterol glycoside to sterol glycoside

SG contains a glucose moiety, which itself contains polar hydroxyl groups. It crystalizes out of the fuel, even at room temperature and relatively low concentration. This crystallization has shown to be problematic as precipitates are known to act as seed crystals for monoglycerides (MG) and diglycerides (DG) [1].

There is currently no ASTM standard to quantitatively analyze for SG in biodiesel. The Cold Soak Filter Blocking Tendency (CSFBT) test, outlined in ASTM D2068, relies on cooling 300 mL of fuel for 16 hours at 4°C. Once brought back to room temperature, the time required to filter the sample through a 0.7 μm glass filter at a pressure differential of 68 kPa is reported. An extensive study of biodiesel precipitates from 4 plants in Europe has shown that there is poor correlation [3] between the filter blocking time and the SG content of the fuel. It has been shown that SG acts as a crystal seed [4] for other compounds, such as MG and DG.

One approach for extracting SG from triglycerides (TG) involves deacylating the oil with 0.1M KOH in methanol for 30 min at 40°C to convert ASG to SG [2]. The reaction is neutralized with 4M HCl, and the methanol is evaporated. Lipids are dissolved in chloroform/methanol (2:1, v/v), and purified by phase partition after the addition of 0.9wt% NaCl in distilled water (Folch wash). After mixing, the top aqueous phase is separated, and the lower organic phase evaporated. The neutral lipids are further fractionated by column chromatography; neutral compounds are eluted with heptane and glycolipids with acetone/methanol (9:1, v/v). The acetone/methanol fraction is then dried and analysed by FTIR or GC-FID to determine purity.

Centrifugation has been used to isolate insoluble material from FAME. Wang [5] isolated SG from soybean, palm and yellow grease based biodiesel by cold soaking for 16 hours at 4°C and reheating to room temperature. The samples were then spun at 5000 G to separate the solids, which were analysed by HPLC after being dissolved in MeOH/CH_2Cl_2 (1:2, v/v). The solids were found to contain 91.1wt% SG. This method was shown to have a detection limit of 100 ppm in FAME and recovery rates range from 75% for B100 containing 500 ppm SG to 99% for B100 with 1.01 wt% SG.

Imperial Oil developed a modified CSFBT test using Isopar K as a solvent to produce B20 solutions [6]. Isopar K is a C_{11}-C_{13} low aromatic hydrocarbon solvent with very low water solubility and a NBP range of 182°C-204°C [7]. This method offers a measure of the partly soluble substances contained in biodiesel and is not a specific analysis for SG.

One approach for the pre-concentration of SG, prior to analysis, is the use of an SPE cartridge equilibrated with heptane. A small sample of biodiesel is introduced in the SPE cartridge and the neutral compounds eluted with heptane. The SG is eluted with a solution of acetone/methanol (9:1, v/v) [8]. This fraction is then dried and analysed by GC-FID. This approach works when distilled biodiesel spiked with SG is used, but fails when untreated biodiesel is used [9]. One explanation for this failure is the presence of a matrix binding SG and other compounds in undistilled biodiesel. When using the SPE procedure with undistilled biodiesel, it is not clear if the SG is not adsorbed by the SPE or if they remain in the SPE cartridge during elution. The net result is the inability to extract and concentrate SG for analysis from undistilled biodiesel.

There are methods to analyze SG content in oil and biodiesel that rely on HPLC with either reverse-phase chromatography with evaporative light-scattering detection [10] or normal phase chromatography with UV detection at 205 nm [11]. The reported detection limit is higher than 30 ppm.

Increasing the amount of sample injected onto the GC column has also been attempted [9]. This direct approach involves the use of 0.5 g of biodiesel (compared to 0.1 g called for in ASTM6584) and dissolving in 2 mL of pyridine. The solution was reacted with 1 mL of BSTFA and analyzed. The total dilution of B100 injected into the GC is 0.5 g B100 in 3 ml of total solvent (pyridine and BSTFA) versus 0.1 g in 8.2 mL (B100:total solvent volume) use in ASTM 6584. This is nearly 12 times the injection concentration used in ASTM 6584. The use of such concentrations on a routine

basis will increase the amount of material deposited in the column requiring frequent column recalibration and reduce useful column life.

The purpose of this study is to analyze SG in biodiesel below 30 ppm, a level which has been found to be problematic [12], with a resolution that makes it possible to quantitatively determine SG. The new technique is to utilize equipment commonly used in routine biodiesel analysis in order to avoid the need for additional analytical equipment.

4.2 Materials and methods

4.2.1 Reactants

Crude non-degummed soybean oil was used to produce SGs from a known source. The oil was stored in an opaque container and shaken before use.

The FAME used in the development of the method was produced from degummed soybean oil stored in an opaque airtight container and shaken before use. Two different biodiesels meeting ASTM 6584 standards were used in the recovery tests. The first was produced from soyabean oil and winterized prior to use in the tests. The second was a canola biodiesel purchased commercially.

Chloroform (0.75 vol% ethanol, ACS grade), methanol (ACS grade), silica gel (60-100 mesh), hexane (ACS grade), n-dodecane (99%, NBP 215°C-217°C, #O2666, LOT #121101, refractive index at 20 °C at 589 nm = 1.4210-1.4230), pyridine (99%), KOH, 37% HCl, N-Methyl-N-(trimethylsilyl) trifluoroacetamide (silylation grade) and sodium methoxide (25wt% in methanol) were all purchased from Fisher Scientific Canada.

Tricaprin was purchased from Chromatographic Specialties (25 mg) and made into an 8 mg/mL solution in pyridine. The SG used in the first recovery test was isolated from crude non-degummed soybean oil using column chromatography and identified by FTIR. The SG used in the second recovery test was purchased from Matreya LLC. (cat #1117, 25 mg steryl glycosides) and dissolved in a 5 mg/mL solution of chloroform/methanol (2:1, v/v).

4.2.2 Instrumentation

GC-FID analysis was used to determine the amount of SG in FAME. In order to transform SG, as well as MG and DG, into more volatile compounds, samples were silylated with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) prior to analysis. A 3800 GC (Varian) GC-FID with a MTX-biodiesel column (Cat # 70291) from Restek Chromatographic Specialties was used. It had a 2 m x 0.53 mm ID retention gap, 15 m x 0.32 mm ID column, and a 0.1 µm film thickness. The method developed for the GC analysis is based on the test method for determination of free and total glycerine in B-100 (ASTM D6584). Calibration for the GC system was achieved by the use an internal standard, tricaprin, and soybean SG isolated from crude soybean oil by column chromatography. The operating conditions for the oven were 50°C for 1 min, 15°C/min to 180^{o}C, 7°C/min to 230°C, 30°C/min to 380°C, hold 10 min. The carrier gas was helium at a constant flow of 3.5 mL/min and the detector was a flame ionization detector (FID) held at 380°C. Cool on-column 1 µL injection volume was used to analyze samples. All chromatographic data was analyzed using the software PeakSimple (SRI Instruments).

ATR-FTIR analysis for solid samples was performed on a Cary 630 FTIR system equipped with a diamond ATR accessory. Data analysis was carried out using the software program Resolution Pro FTIR (Agilent Technologies). All spectra were obtained using 140 background scans and 140 samples scans at a resolution of 2 cm^{-1}. Enough sample material was used to completely and

evenly cover the diamond window of the ATR accessory, and the liquid was pulled by capillary force using the built in compression-dial. The solvent system was also analyzed and its spectrum subtracted from liquid sample analysis to obtain the compound IR spectrum.

Column chromatography was performed using a 38 mm ID x 178 mm glass column equipped with a stopcock. The silica gel was activated at 120°C for 8 hours in a convection oven, cooled and stored in a desiccator until needed. The chloroform was passed through a bed of activated silica gel to remove the 0.75 vol% ethanol added as a preservative before used. The glass column was plugged with Pyrex glass wool before loading with silica gel. Roughly 2 mm of inert sand was added to the top of the silica bed to dampen disturbances from pouring the solvents.

Temperature controlled centrifugation was carried out using a Hermle Z400K centrifuge equipped with a Hermle 220.97 rotor (28°angle, max radius 9.5 cm and a maximum speed of 6000 rpm equivalent to 3820 xg). The centrifuge was cooled for 30 min prior to use.

In the final centrifugation step, an IEC HN-SII centrifuge equipped with a horizontal 958 swing-type rotor spun was used at a speed of 2000 rpm. A clearly defined pellet was obtained from fine solids dispersed in the final n-dodecane wash using this type of rotor.

4.2.3 Methods

4.2.3.1 Isolation and purification of sterol glycosides from crude soybean oil

Deacylation of ASG in crude soybean oil

In order to convert ASG to SG, 20 mL of crude soybean oil, in a polypropylene centrifuge tube, was reacted with 20 mL of 0.1M KOH solution in methanol for 30 min at 40°C. The reaction was stopped by adding a 4M HCl solution in methanol until neutralized. The solution was spun at 2000 rpm for 20 min, and the methanol removed by evaporation.

Folch wash

Lipids from the deacylated oil solution were dissolved by adding 30 mL of chloroform/methanol (2:1, v/v), and vortexing for 5 min. The solution was then centrifuged at 6000 rpm for 45 min, separating cell debris from the dissolved lipids. The supernatant was taken up by suction and washed with 7.5 mL of 0.9 wt% NaCl in deionized water. After vortexing for 5 min, the solution was centrifuged at 2000 rpm for 15 min, and a two-phase system developed. The top polar phase was removed via suction, and the organic phase was dried in a rotary evaporator at 350 mbar, at 50°C for 4 hours.

Column chromatography

Column chromatography was employed to fractionate the lipids extracted in the organic phase of the Folch wash. A silicic acid column was prepared by first activating 80 g of silica gel at 120°C for 8 hours in an oven. This corresponded to a loading rate of 80 g silica gel for 1 g of crude lipid extract. The silica gel was cooled in a desiccator and equilibrated with hexane. It was then placed in a 4 cm x 38.5 cm glass column plugged with glass wool. The isolated lipid extract was mixed in heptane and carefully poured onto the column without disturbing the silica gel bed. The column was eluted with 100 mL of heptane to remove neutral lipids, then with 100 mL of acetone/methanol (9:1, v/v) to remove glycolipids; including SG. The acetone/methanol fraction was dried in a rotary evaporator at 350 mbar, for 4 hours at 50°C, which produced an off-white waxy substance. This isolate was dissolved in chloroform/methanol (2:1, v/v) and stored in a refrigerator to prevent degradation.

4.2.3.2 SG recovery studies

A winterized soybean biodiesel and a commercial canola biodiesel were tested in the SG recovery studies. The soya biodiesel was produced by reacting soybean oil with methanol and sodium methoxide. The MeOH:Oil ratio and catalyst concentration on an oil basis were: 6.5:1, 0.7 wt%. The FAME produced was neutralized, water washed and evaporated. The biodiesel was winterized at -8°C for 15 hours and centrifuged to remove waxes and glycerides. The SG used in this recovery test was isolated from crude soybean oil by column chromatography.

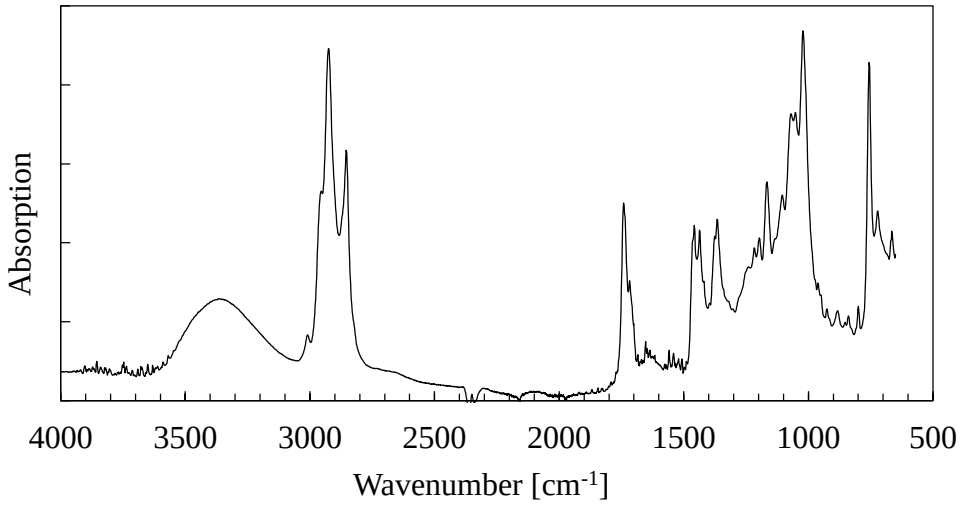
The second was a commercial grade biodiesel produced from canola oil (Milligan Biofuels, SK) labeled canola-biodiesel. The SG used in this recovery test was purchased from Matreya LLC.

4.3. Results and discussion

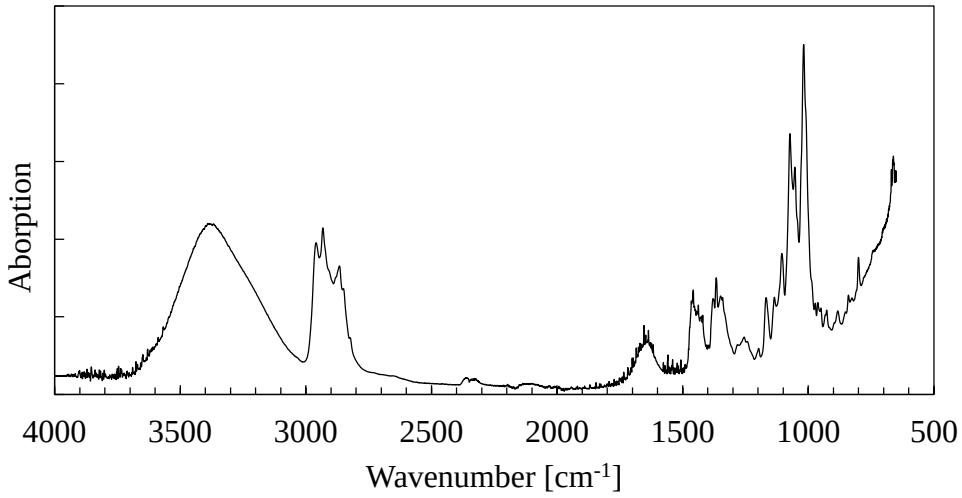
4.3.2 Characterization of precipitates from soya oil

FTIR of acetone/methanol fraction

The acetone/methanol fraction isolate was analysed using FTIR to obtain its absorption spectrum. Using a diamond ATR accessory, 20 µL of dissolved isolate was placed on the crystal lens. A full spectrum analysis was obtained, which can be found in Figure 4-2(a). A commercial standard (Matreya Inc, cat #1117) was later analyzed by FTIR, and its spectrum is found in Figure 4-2(b).



(a)



(b)

Figure 4-2 **(a)** FTIR spectrum of the substance in the acetone/methanol (9:1, v/v) fraction

(b) FTIR spectrum of commercial sterol glycoside sample (98 +% purity)

The dried acetone/methanol fraction exhibits the same absorption spectra as the commercial SG standard. The absorption peaks at 1750 cm^{-1} and 2920 cm^{-1} in Figure 2(a) are indicative of C = O

and C – C bonds, caused by residual oil in the isolate. Further purification was not needed, as the residual oil was not detectable by GC-FID (Figure 4-3).

GC-FID analysis of soybean oil SG

The GC chromatogram of SG isolated from soybean oil was obtained by GC-FID using the same parameters outlined in ASTM D6584. The equivalent of 0.5 mg SG was transferred into a glass vial, and the solvent was evaporated using a light stream of nitrogen. After drying, 100 µL of tricaprin standard (8 mg/mL pyridine) and 100 µL of MSTFA were added, and left to react for 45 min at room temperature. The solution was diluted in 8 mL of anhydrous heptane and mixed. The sample was transferred to a GC vial, and a 1 µL injection volume was used for analysis. The chromatogram of SG isolated from soybean oil can be found in Figure 4-3.

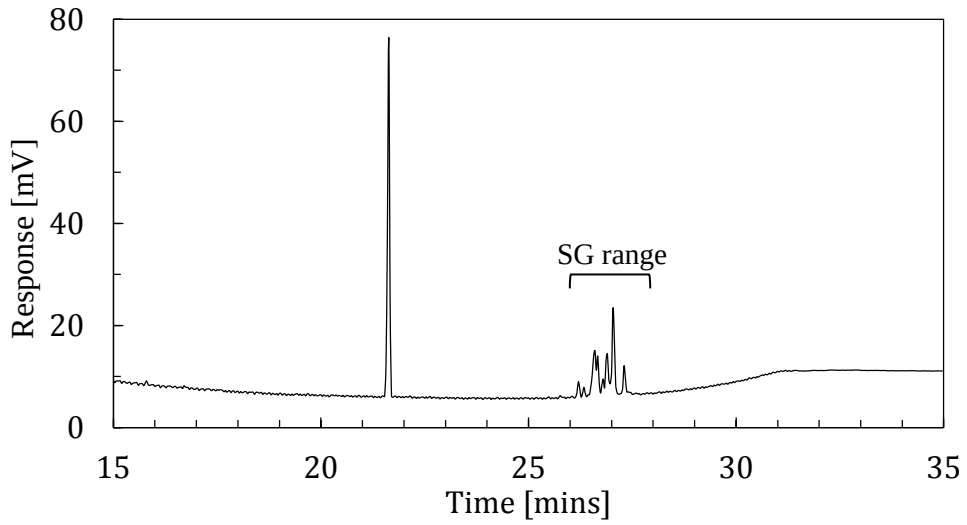


Figure 4-3: GC-FID chromatogram of SG isolated from soybean oil

This chromatogram shows a clean tricaprin peak at 21.6 min and multiple SG peaks at 26-28 min, or a relative retention time of 1.2-1.3 with respect to tricaprin. It does not indicate the presence of TG (30+ min), which confirms the SG purity of the isolate.

4.3.3 Quantitative analysis of SG in biodiesel

Selection of a decompatibilization solvent

The following section discusses the isolation of SG from biodiesel. The ideal solvent to perform this task has low aromaticity, low viscosity, and high hydrophobicity. A search for a generic aliphatic compound to affect the precipitation was performed. Water is sparsely soluble in hydrophobic solvents; its insolubility was used as a measure of hydrophobicity. The melting point and water solubility of various n-alkanes were plotted vs their carbon number in Figure 4-4.

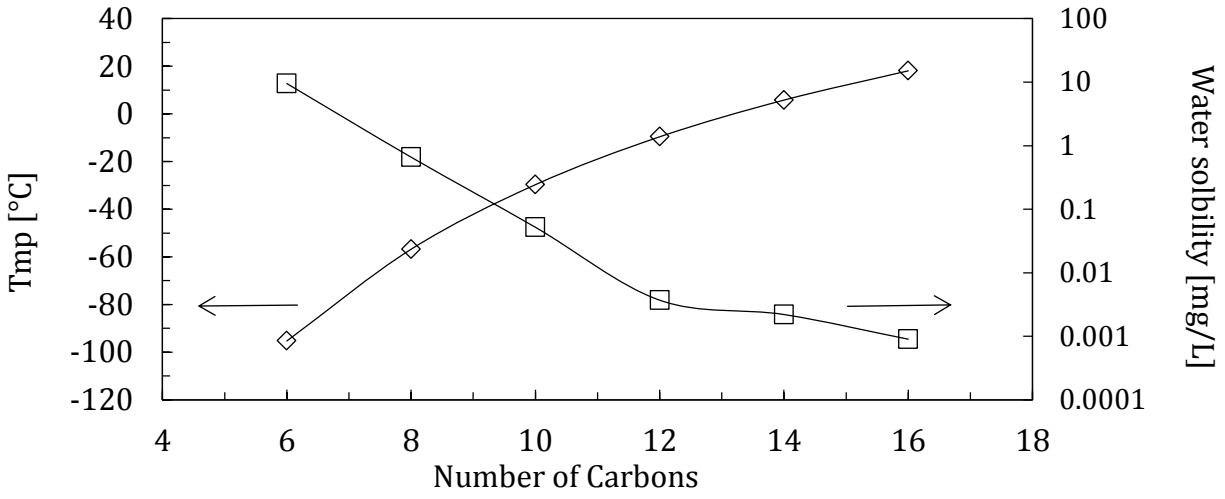


Figure 4-4 Melting point [^{o}C] and water solubility [mg/L] vs carbon number **[37]**

N-heptane has a very low melting point, but high water solubility, while cetane has very low water solubility, but a high melting point. The solubility of water decreases considerably up to C_{12} then levels off beyond. The most useful aliphatic hydrocarbon for decompatibilization of SG in FAME was found to be n-dodecane (C_{12}). It has a melting point of -9.6°C, and a water solubility of 0.0037 mg/L. These properties ensure that the SG is decompatibilized in a liquid solution, and can be removed by temperature-controlled centrifugation. Its hydrophobicity ensures that very little SG is dissolved with the addition of the solvent. The narrow boiling point of the n-dodecane

used ensured its carbon number and hydrophobicity. This grade of n-dodecane is readily available from several suppliers.

4.3.4 Method development: Isolation of precipitates from FAME

The procedure for the isolation of SG from biodiesel can be found in Figure 4-5 below. The steps are detailed as follows:

1) Prepare a 30 mL solution of 1:1 (vol/vol) FAME:dodecane by carefully weighing both liquids, and determining their corresponding volume by density.

2) Acidify the mixture using 15 µL (approx. one drop) of concentrated HCl then vortex for 2 min.

3) Cold soak the mixture in an ethylene glycol bath at -8°C for 16 hours. Waxy solids should form.

4) Centrifuge at -8°C, 6000 rpm for 45 min. Discard the supernatant leaving behind a pellet of precipitate.

5) Dissolve precipitates in 10 mL of chloroform/methanol (2:1, v/v) mix and add 2 mL of 0.9 wt% NaCl in distilled water.

6) Remove top phase (aqueous phase) by pipette, and dry the organic phase over a stream of nitrogen. Strip all water droplets from the organic phase by impinging nitrogen on its surface. (Sample A fig 4-5).

7) Wash dry solids with 5mL of n-dodecane, and centrifuge with a variable-angle centrifuge at 2000 rpm for 45 min (Sample B Fig 4-5).

8) Remove n-dodecane by pipetting, leaving a clearly defined pellet at the bottom of the tube.

9) Analyze solids by GC-FID.

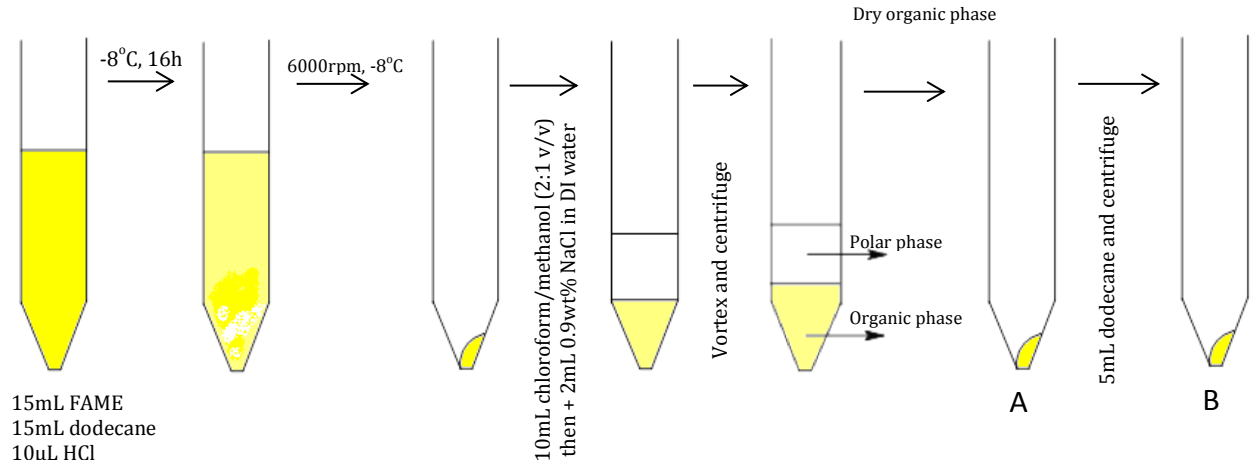


Figure 4-5 Schematic for SG extraction and purification

Trace residual catalyst in the FAME sample was found to affect the Folch liquid-liquid extraction. This protonated the SGs and made them soluble in the organic phase of the Folch wash. When no HCl was added to the B50 samples, the pH of the aqueous phase during the Folch wash was measured to be approx. 10, using pH paper. The detection of SG in the organic phase was not possible at this higher pH. When the acid was added, the pH of the aqueous phase was measured to be approx. 4; SG was detected in the organic phase at this lower pH.

4.3.5 Analysis of FAME insolubles

To study the effects of the final n-dodecane wash, the solids from centrifuge tube A (Figure 6) were analyzed by GC-FID. They were dissolved and transferred to a 20 mL reaction glass vial. After drying over nitrogen gas, 100 µL of tricaprin standard and 100 µL of MSTFA were added. The content was left to react for 45 min at room temperature. After silylation, 4 mL of heptane was added and 1.5 mL of this solution was transferred to a glass GC vial for analysis. The resulting GC-FID chromatogram can be found in Figure 4-6 below. It shows a large concentration

of MG and DG. Increasing the injection concentration of solids would increase column loading, decreasing the useful life of the column. A second wash with 5 mL of n-dodecane completely removed the interfering MG, DG and TGs as seen in Figure 4-7. This permitted the use of 4 mL of heptane in the final sample dilution instead of the 8 mL called upon in ASTM D6584. The small derogation increased the detection limit of the method by a factor of 2.

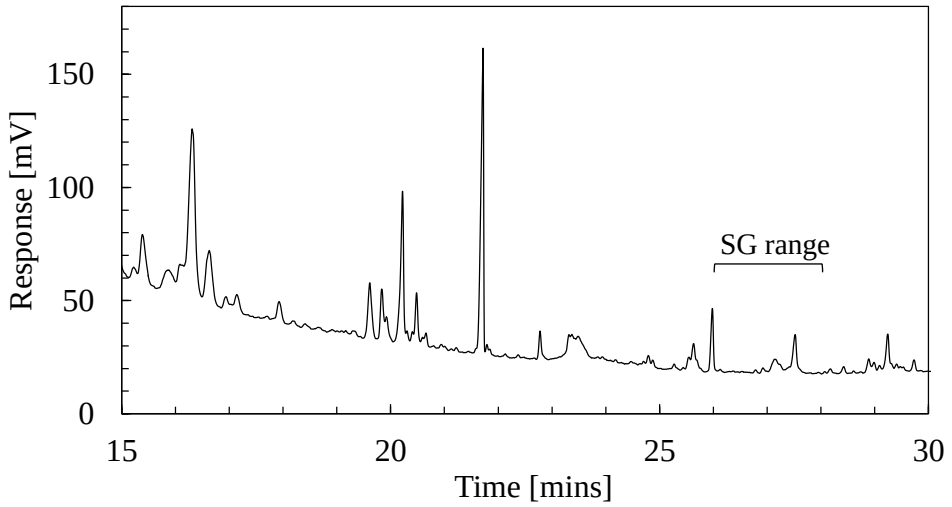


Figure 4-6GC-FID chromatogram of biodiesel solids without final n-dodecane wash (Sample A)

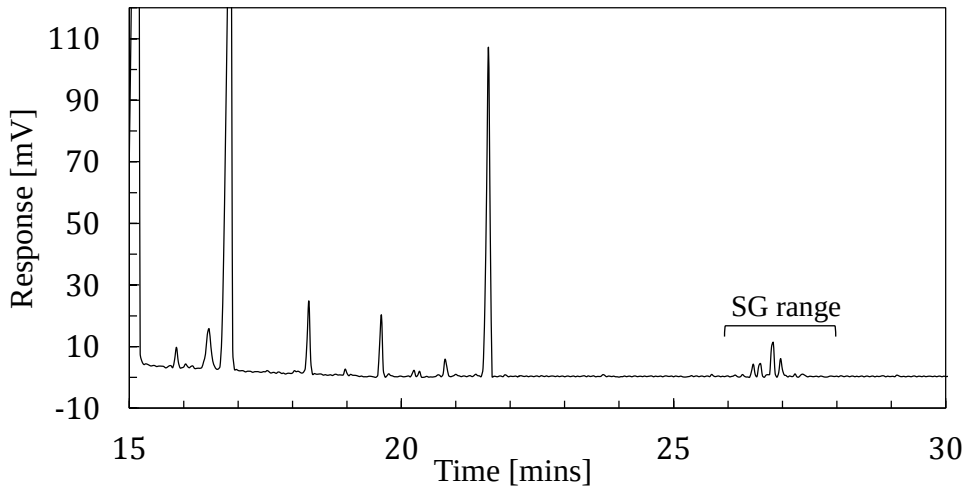


Figure 4-7GC-FID chromatogram of biodiesel solids washed with 5mL n-dodecane (Sample B)

4.3.6 Calibration curve

In order to quantify the SG response of the GC-FID, a calibration curve was produced from the 5 mg/mL SG standard solution. Specific quantities of this solution (10, 20, 40, 70 and 100 µL) were dried in a glass vial using a light stream of nitrogen. After drying, 100 µL of tricaprin standard and 100 µL of MSTFA were added to the vial, and the contents left to react for 45min. The solution was diluted in 4 mL of heptane and the sample was analyzed by GC-FID. The calibration curve used in these experiments can be found in Figure 4-8 below. The r^2 value in the calibration curve meets the level of 0.99 or greater for reference component analysis set by ASTM 6584 and can be used in the quantitative analysis of SG.

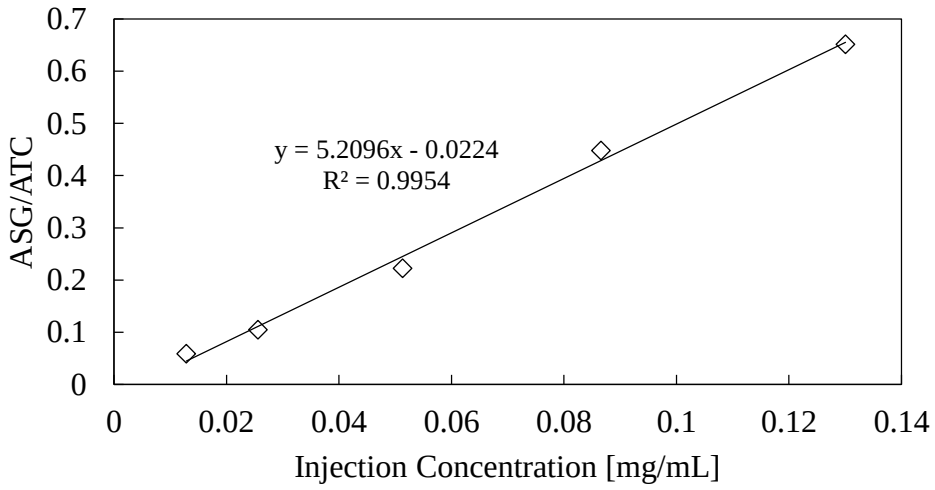


Figure 4-8: Soybean SG calibration curve for GC-FID analysis based on the sum of the peaks observed for retention times between 1.2 and 1.3 relative to tricaprin.

The chromatogram of SG produces multiple peaks, and first order polynomial showed to have an excellent fit using multiple peak integration. Multiple peak integration has also been used in HPLC analysis of SG and found to offer a good correlation [11]. The lowest value in the calibration curve corresponds to the detection limit of the method. If 100% of the SG were to be extracted from a 15 ml biodiesel sample using the method described above, the value of 0.0128

mg SG/mL heptane injected would correspond to a concentration of 1 ppm SG in the biodiesel. It is important to note here that the detection limit will depend on the ability of the method to extract SG from biodiesel.

4.3.7 Recovery test

To validate the extraction method illustrated in Figure 5, soy and canola biodiesels were spiked with known quantities of SGs, and the recovery rates were calculated based on the quantity of SGs detected by GC-FID.

SG Recovery from Soy biodiesel

Pure soybean biodiesel was obtained by first winterizing at -8^{o}C for 16 hours then centrifuged to remove MG, DG and SG. The supernatant was analysed using the procedure described above. This permitted the evaluation of the reproducibility of the method in very pure biodiesel. A known quantity of SG (0.5 mg) was added to 3 centrifuge tubes and the solvent (chloroform/methanol) removed using nitrogen. Biodiesel was then added to these tubes and vortexed for 5 min. The SG concentration in the spiked samples was determined by the method described in sections 4.3.4 and 4.3.5 above. Triplicate samples were spiked with SG and the recovery rates are reported in Table 4-1 below.

Table 4-1: Recovery rates using winterized soy-biodiesel

Name	Weight biodiesel [g]	SG added [mg]	SG in sample by GC [mg in sample]	SG (sample-control) [mg]	Recovery
Control	13.02	0	0	0	-
Sample 1	13.15	0.5	0.491	0.491	98.1%
Sample 2	13.55	0.5	0.497	0.497	99.5%
Sample 3	13.08	0.5	0.494	0.494	99%

The results show that all the SG was extracted in the cold-soaked soybean biodiesel. This is consistent with claims that SG extraction is feasible using very pure biodiesel.

SG Recovery from Canola biodiesel

Another recovery test was performed using commercial canola-biodiesel at a cold soak temperature of -8°C. The samples were spiked using the same method described above, and the solids were extracted using the method described in Figure 4-5, and analyzed by GC-FID. A control sample was also analyzed and found to contain 15 ppm of SG. The results of the recovery test using commercial canola-biodiesel can be found in Table 4-2 below.

Table 4-2: SG recovery test using commercial canola-biodiesel at -8°C

Name	Weight biodiesel [g]	SG added [mg]	SG in sample by GC [mg in sample]	SG recovered [mg]	Recovery [%]
Control	12.38	0	0.1714	0	-
Sample 1	13.12	0.5	0.6867	0.505	101%
Sample 2	13.01	0.5	0.7081	0.528	106%
Sample 3	13.71	0.5	0.6924	0.5026	101%

The recovery test was also performed using the same canola biodiesel, but with a cold soak temperature of -15°C instead of -8°C. The results of this recovery test can be found in Table 4-3 below.

Table 4-3: SG recovery test using commercial canola-biodiesel at -15°C

Name	Weight biodiesel [g]	SG added [mg]	SG in sample by GC [mg in sample]	SG recovered [mg]	Recovery [%]
Control	13.12	0	0.2156	-	-
Sample 1	13.07	0.5	0.6783	0.464	93%
Sample 2	13.13	0.5	0.7207	0.505	101%
Sample 3	13.45	0.5	0.7188	0.498	100%
Sample 4	13.73	0.5	0.7239	0.498	100%
Sample 5	13.58	0.5	0.7166	0.493	99%

The lower temperature resulted in a higher formation of partially soluble material, which facilitated the separation. The recovery rates of this test are similar to those of canola biodiesel at -8°C, but centrifugation at -15°C was more successful with the higher amount of MG and

saturated esters precipitated. Note that these additional precipitated substances were removed in the dodecane wash included in the method.

Overall, the recovery of SG was found to be 99% for winterized soy with a standard deviation of 0.7 % and 100% with a deviation of 3.5 % for canola. The sensitivity of this method allows for the quantifiable detection of SG in the sample. If a greater resolution is required, the injection concentration could be increased as the new method eliminates MG, DG and TG from the sample. If present, these compounds could load the GC column and reduce its useful working life.

4.4Conclusion

SG precipitates have been shown to cause problems with filter plugging and long term storage of biodiesel, which can limit its use as a renewable substitute for conventional diesel fuel. SG has been shown to interact with other compounds in FAME, resulting in a difficult or incomplete extraction. The present study was successful in measuring SG content of biodiesel with a detection limit of 1 ppm using existing equipment found in biodiesel quality analysis laboratories. A linear calibration curve (R^2=99) was produced using multiple peak integration of SG peaks. The detection limit could be further reduced by changing sample size or dilution ratio prior to injection, as very little TG is present when samples are washed with n-dodecane.

This confirms that SG can be fully decompatibilized from biodiesel using the new method proposed in this work allowing for the quantitative determination of SG in biodiesel without specialized equipment. The non-reliance on a manufactured product such as a filtration device or SPE sorbent, the low detection limit and high reproducibility of the new analytical method could form the basis of an ASTM standard for SG analysis in biodiesel.

Chapter 5

The Reduction of Sterol Glycosides during the Transesterification of Soybean Oil to Improve Biodiesel Cold Flow Properties

A. Montpetit and A.Y. Tremblay, Department of Chemical and Biological Engineering, Univ. of Ottawa (to be submitted)

5. The Removal of Sterol Glycosides during the Transesterification of Soybean Oil to Improve Biodiesel Cold Flow Properties using Ultra-Filtration

Abstract

The presence of sterol glycosides (SG) in biodiesel is known to affect the cold flow properties and operability of the fuel. In cold temperatures, or when stored for extended periods of time, SG can precipitate out of solution and act as a crystal seed for other compounds. They are currently removed either by distillation, or by cold-filtration; both of which increase production costs. The purpose of this work is to determine the ability of a ceramic membrane to separate SG from a reactive Fatty Acid Methyl Ester (FAME) mixture, while biodiesel is being produced; without cooling or the use of distillation. The separation of SG across the membrane also gave information about the concentration of solubilized SG in the FAME.

This was achieved by producing biodiesel from degummed soy oil and filtering the reactive mixture through a ceramic membrane. The conditions studied were: catalyst concentrations of 0.3wt%, 0.5wt% and 0.7wt%, MeOH:Oil ratios of 4:1, 5:1, 6:1, 7:1 and 9:1. SG content from both the retentate and permeate streams were precipitated and analysed by GC-FID. The separation factor of SG was determined and used to study the solubility of SG in the reactive FAME mixture. All analyses were performed on FAME phases that were neutralized, methanol evaporated but not washed.

It was observed that the highest separation for SG (86 %) was obtained when the reaction conditions were 0.7 wt% and 4:1 MeOH:Oil ratio. It was the lowest (0%) at 0.3 wt% and 4:1 MeOH:Oil ratio, where unreacted feed components and intermediates such as MG and DG were found to solubilize SG in the reactive mixture.

Deprotonation of the hydroxyl groups on SG was found to decrease the solubility of SG in the reactive FAME phase. The solubility of SG in the FAME phase increased with the MeOH:Oil ratio used in the experiment. This was evaluated in relation to the Hildebrand solubility parameter of the reactive FAME mixture. As the fraction of methanol in the FAME phase increased, the polar and hydrogen bonding components of the FAME mixture increased and partially solubilized SG.

SG was found to be more soluble in poorly reacted FAME and when methanol was used in excess, and less soluble in highly reacted FAME and when the catalyst concentration in the glycerol phase was high enough to partially deprotonate SG. The use of a membrane separation process during the transesterification reaction was highly beneficial in separating SG from biodiesel.

Keywords: Sterol glycosides, Biodiesel, FAME, Purification, Solubility,

4.1 Introduction

Biodiesel is a renewable alternative to conventional petroleum diesel. It is produced by the transesterification of vegetable oils or animal fats using short chain alcohols in the presence of a catalyst. Poor conversion resulting in partially reacted triglycerides can affect the cold temperature operability of the fuel. Other minor components can also affect the stability of the fuel such as plant sterols. Acylated sterol glycosides (ASG) are an important class of natural compounds that are part of plant cell wall and are fully soluble in oil. Although feedstock oil is degummed before being transesterified to biodiesel, these compounds are never fully removed and are still found in the finished fuel.

During the transesterification process, ASG are converted to SG, as seen in Figure 5-1. SG is insoluble in biodiesel and will precipitate over time decreasing the fuel's cold weather operability. SG is known to act as a seed for the crystallization of minor components found in biodiesel such as Monoglycerides (MG) and diglycerides (DG) [28].

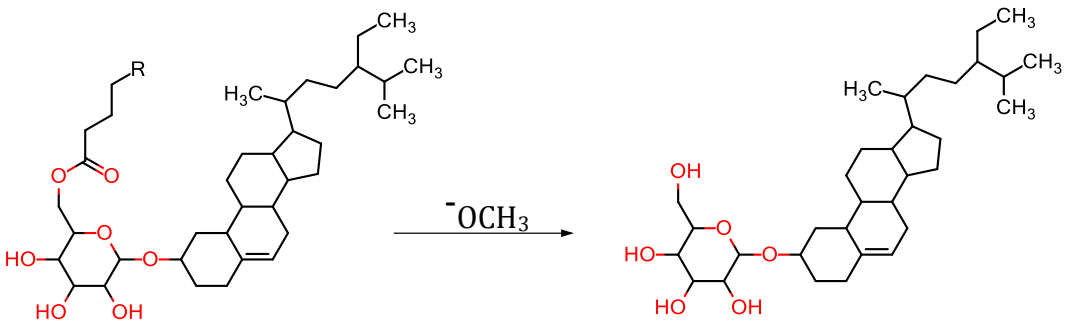


Figure 5-1: Deacylation of acylated sterol glycoside to sterol glycoside

Several attempts at reducing sterol glycoside levels in finished biodiesel have been performed. Enzymatic removal [37] has been shown to reduce SG levels by 81% with the addition of a synthetic codon-optimized version of the LacS gene expressed from E. Coli. This enzyme is water

soluble so the hydrolysis of SG is mass transfer limited between the aqueous phase and biodiesel phase. The addition of a surfactant, polyglycerol polyricinoleate (PGPR), was found to increase the hydrolysis rate from 8% to 81%. This surfactant is partially soluble in biodiesel which may pose additional problems. This can be solved as the enzyme is stable over a large range of pHs and could be added to the water washing process. Filtration through diatomaceous earth [24] has also been used to reduce SG levels in finished biodiesel. This was accomplished by cooling the finished biodiesel to precipitate minor components and filtering through fine diatomaceous earth. This has been shown to not only reduce levels of SG in finished fuel, but MG and DG as well. The main issue with this method is the amount of energy needed to cool a large volume of finished biodiesel. Finally, distillation has been shown to produce very pure biodiesel ester devoid of any minor components [1] but like cooling, increases production costs. Synthetic membranes have been used in a reactive biodiesel environment to enhance the transesterification reaction [19].

The purpose of this paper is to study the effect of ultrafiltration on the separation of SG with the aim of removing SG from unwashed biodiesel (FAME) without cooling or distillation. The separation was performed on reactive FAME mixtures in the presence of methanol. This has the advantages of reducing the viscosity of the feed mixture which leads to an increased permeation flux across the membrane at a given pressure.

5.2 Materials and methods

5.2.1 Reactants

Degummed soybean oil was used in the production of FAME. The oil was kept in an airtight opaque container and shaken before use. Chloroform (0.75% ethanol, ACS grade), methanol (ACS grade), n-dodecane (99%, NBP 215°C-217°C), pyridine (99%), HCl, N-Methyl-N-

(trimethylsilyl) trifluoroacetamide (silylation grade) and sodium methoxide (25wt% in methanol) were purchased from Fisher Scientific Canada.

Monoolein, diolein, triolein and tricaprin GC standards were purchased from Chromatographic Specialities Inc. Stock solutions and calibration curves for MG, DG, TG and tricaprin were produced according to ASTM D6584. SG standard was purchased from Matreya Inc. (25mg, Catalogue #1117).

5.2.2 Instrumentation

GC analyses were used to determine the amount of SG, MG, DG and TG in FAME produced in this work. In order to transform SG, as well as MG and DG into more volatile compounds, samples were sylilated with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) prior to analysis.

Characterization of MG, DG and TG was performed according to ASTM D6584. A 3800 GC (Varian) with a Restek MTX-Biodiesel column from Chromatographic Specialties (Brockville, ON) with a 2 m, 0.53 mm retention gap and a 15 m, 0.32 mm, 0.1 µm film thickness was used. The GC-FID analysis is based on the test method for the determination of free and total glycerine in B-100 (ASTM D6584). Calibration of the GC system was achieved by the use an internal standard, tricaprin, and various amounts of SG, MGs, DGs and TGs. The operating conditions for the oven were 50°C for 1 min, 15°C/min to 180°C, 7°C/min to 230°C, 30°C/min to 380°C, hold 10 min. The carrier gas was helium at a constant flow of 3.5 mL/min, the detector was a flame ionization detector (FID) held at 380^{o}C, cool on-column injection with a sample size of 1µL was used. All chromatographic data was analyzed using the PeakSimple software (SRI Instruments).

Temperature controlled centrifugation was carried out using a Hermle Z400K centrifuge, equipped with a Hermle 220.97 rotor (28° fixed angle, max radius 9.5cm and a max speed of 6000 rpm, equivalent to 3820 xg). The centrifuge was cooled to -8°C for 30 min prior to use.

Variable-angle centrifugation was carried out with a variable angle centrifuge using an IEC HN-SII centrifuge equipped with a horizontal 958 swing-type rotor. A clearly defined pellet was formed at the bottom of the centrifuge tube using a swing-type rotor. The isolation of the fine solids dispersed in n-dodecane was more effective using this type of centrifuge.

5.2.3 Production of FAME

A tubular TiO_2 300 kD MWCO membrane was used to filter the FAME produced from degummed soy oil. The experimental parameters described in Table 5-1 were tested.

Table 5-1: Experimental parameters

Catalyst (wt% on oil basis) - MeOH:Oil ratio				
0.3wt% oil - 4:1	0.3wt% oil - 5:1	0.3wt% oil - 6:1	0.3wt% oil - 7:1	0.3wt% oil - 9:1
0.5wt% oil - 4:1	0.5wt% oil - 5:1	0.5wt% oil - 6:1	0.5wt% oil - 7:1	0.5wt% oil - 9:1
0.7wt% oil - 4:1	0.7wt% oil - 5:1	0.7wt% oil - 6:1	0.7wt% oil - 7:1	0.7wt% oil - 9:1

The reactor consisted of a three-neck flask heated by a hot water bath, a condenser and thermometer, as seen in Figure 5-2. The FAME was left to react for 60mins, after which the recirculation pump was turned on while keeping the mixture temperature at 60°C. The permeate stream was collected in a graduated cylinder and a cross-membrane pressure of 40-50 psi was applied. After 100 mL was collected, the rest of the reactive FAME mixture was placed in a separatory funnel for 60 min held at 50°C by a hot water bath. The glycerol was separated from

the retentate FAME by gravity settling, and both FAME streams were neutralized using concentrated HCl. After neutralization, both samples were dried in a rotary evaporator at 350 mbar, 60°C for 3 hours to remove excess methanol.

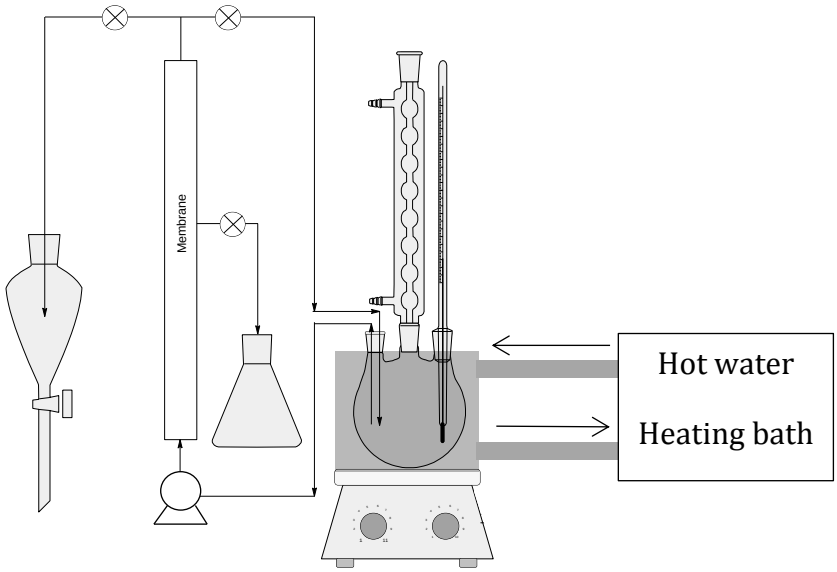


Figure 5-2: Reactor with membrane filtration

Isolation and analysis of SG from FAME

SG were decompatibilized from the methanol evaporated FAME by the addition of n-dodecane (NBP 215-217°C, containing <30 ppm aromatic) in a 1:1 volume ratio. In this step, 13 g (15 mL) of FAME was added to a 50 mL polypropylene (PP) centrifuge tube and diluted with 13 g (15 mL) of n-dodecane. The solution was acidified by adding 10 µL of concentrated HCl. The mixture was vortexed and placed in a -8°C ethylene glycol/water bath for 16 hours to precipitate the SG. The chilled samples were centrifuged at 6000 rpm for 45 min at -8°C to isolate precipitates. The clear liquid was decanted, leaving behind a clearly defined solid pellet. A liquid-liquid Folch wash was used to separate polar compounds from neutral compounds. The pellet was dissolved in 10 mL of chloroform/methanol, and 2 mL of 0.9wt% NaCl in distilled water was added to the

mixture. After vortexing for 2 min, the solution was left to settle and 2 phases formed. The top aqueous phase contained the polar components and the bottom organic phase contained the neutral organic compounds, including SG. The top phase was pipetted and discarded, and the bottom phase dried over a light stream of nitrogen. To remove DG and TG, the dried solids were washed with 5 mL of n-dodecane. The mixture was centrifuged at 2000 rpm for 30 min in a variable angle centrifuge. The n-dodecane supernatant was carefully pipetted, leaving behind a fine white suspension. A schematic of the extraction method can be found in Figure 5-3.

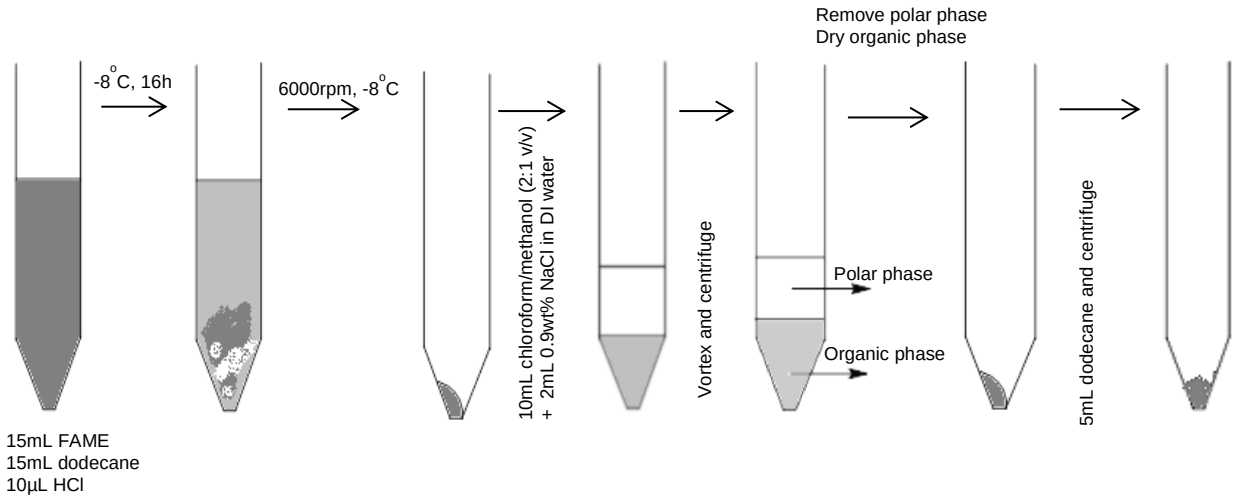


Figure 5-3: Schematic for SG extraction and purification

The extracted solids were dissolved in chloroform/methanol (2:1, v/v) and transferred from the PP centrifuge tube to a glass sample tube. The PP tube was then rinsed 3 times with chloroform/methanol, and collected in the glass tube. The solution was dried over a light stream of nitrogen gas to remove the solvent. The dried solids were sylilated with 100 µL of MSTFA and 100 µL of tricaprin (8 mg/mL in pyridine) was added as an internal standard. The solution was left to react for 45 min at room temperature. After sylilation, the sample was diluted in 4 mL of anhydrous heptane and analyzed by GC-FID using a 1 µL injection volume. Analysis of SG was

performed using a heptane dilution of 4 mL instead of 8 mL called for in ASTM D6584. This resulted in a lower detection limit for SG and a higher resolution.

3. Results and discussion

5.3.1 Calibration curve

In order to determine the concentration of SG in the FAME samples; a calibration curve was produced using the commercial SG standard purchased from Matreya LLC. (25 mg steryl glycosides, cat #1117). It was dissolved in a 5 mg/mL solution of chloroform/methanol (2:1, v/v). Specific volumes of this standard solution (100 µL, 70 µL, 40 µL, 20 µL and 10 µL) were pipetted in a glass vial and dried using a light stream of nitrogen gas. After drying, 100 µL of the tricaprin standard was added and the solids were sylilated with 100 µL of MSTFA. The solution was left to react for 45 min, and then diluted with 4 mL of anhydrous heptane. This solution was analyzed by GC-FID using a 1 µL injection volume. The tricaprin was detected at 21.5 min and SG at 26-28 min. The area from RRT= 1.21-1.3 was measured using multiple peak integration and divided by the area of the tricaprin internal standard. The relative area was plotted against the injection concentration to produce the calibration curve found in Figure 5-4

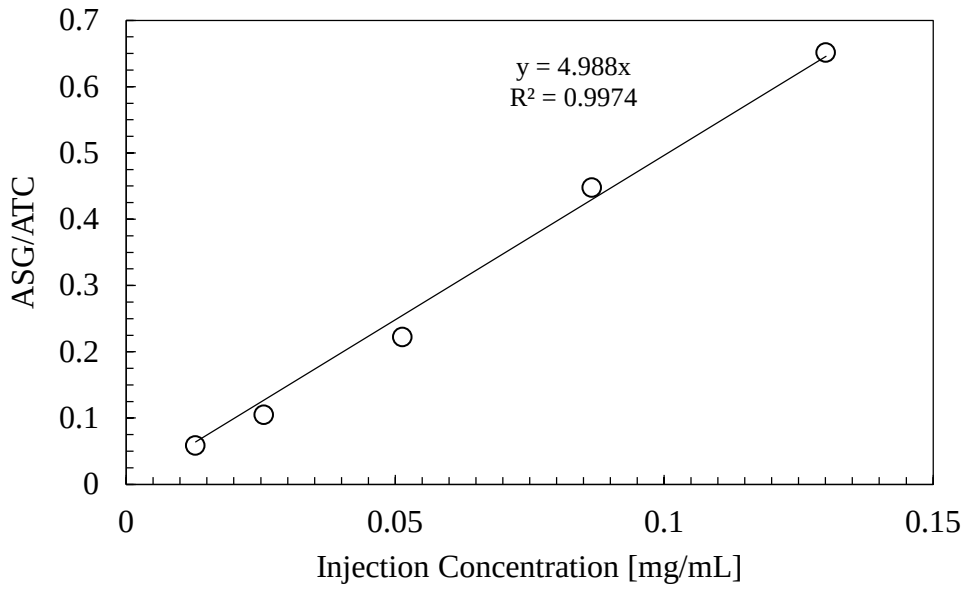


Figure 5-4: Sterol glycoside GC-FID calibration curve

In order to demonstrate the resolution of the extraction method, two chromatograms can be compared: Figure 5-5 for FAME produced from 9:1 MeOH:Oil and 0.7wt% $NaOCH_3$ and Figure 5-6 for the solids extracted and analysed using the method described above. The retention times for all components are the same in both cases as the samples were both analyzed on the same column using the temperature program specified in ASTM 6584. In Figure 5-5 FAME was analysed directly as specified in ASTM 6584. In Figure 5-6 the sylilated solids extracted from 15 mL of FAME were diluted in 4 mL of heptane prior to GC injection instead of 8 mL specified in ASTM 6584. All other analysis conditions were as specified in ASTM 6584.

The amount of substance detected between 26-28 min in Figure 5-5 is not detectable indicating that the direct analysis of biodiesel using ASTM 6584 cannot be used to quantitatively determine SG.

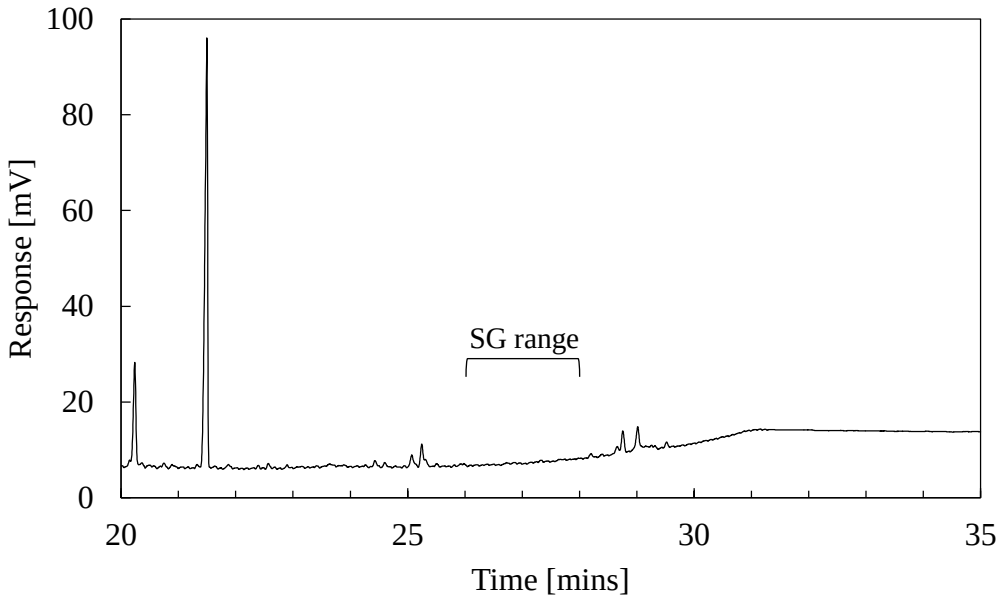


Figure 5-5: Chromatogram of the direct analysis of FAME produced from degummed soybean oil using 9:1 MeOH:Oil and 0.7wt% catalyst

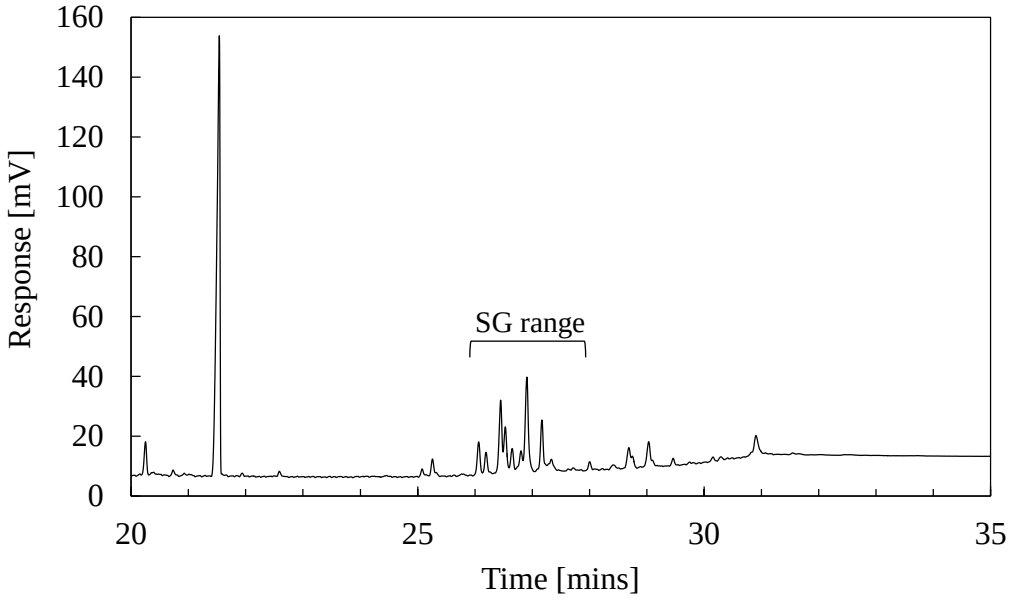


Figure 5-6: GC-FID chromatogram of solids extracted from the retained FAME produced using 9:1 MeOH:Oil and 0.7wt% catalyst

5.3.2 Sterol glycoside separation

The SG concentration in the membrane retentate and permeate for all FAMEs produced using experimental conditions listed in Table 5-1 were determined. The separation factor (SF) for each run was calculated using Equation 2 below;

$$SF = 1 - \frac{[SG]_{permeate}}{[SG]_{retentate}} \quad \textbf{(1)}$$

The separation factor for each run was plotted against the MeOH:Oil ratio, found in Figure 5-7.

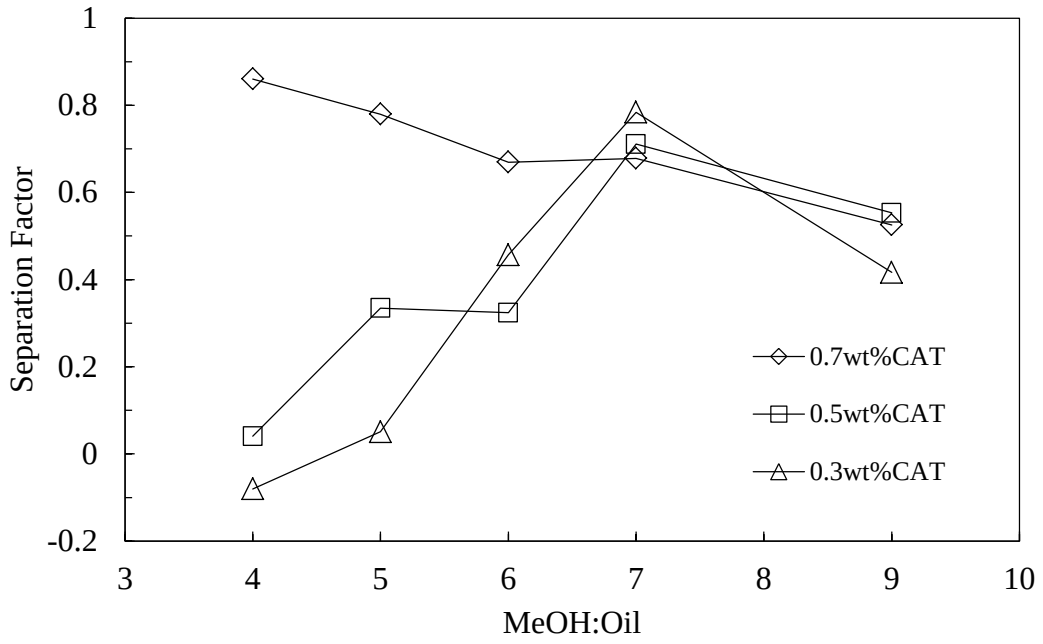


Figure 5-7: Sterol Glycoside Separation Factor vs MeOH:Oil ratio

The separation factor of FAME produced using low catalyst concentrations (0.3 wt % and 0.5 wt %) is seen to increase with increasing MeOH:Oil ratio, until it reaches a maximum at 7:1 MeOH:Oil. It then drops when the MeOH:Oil ratio increases to 9:1.

The separation factor in the FAME produced using 0.7 wt% catalyst is highest (86%) at 4:1 MeOH:Oil ratio then decreases to 53% at a MeOH:ratio of 9:1. These trends will be explained in the following sections.

5.3.3 Characterization of unreacted components

In order to fully characterize the FAMEs produced in these experiments; the MG, DG and TG in the samples were determined according to ASTM D6584. Samples containing high levels of unreacted TG were further diluted with anhydrous heptane to decrease GC column loadings. The total conversion of saponifiable matter was calculated from the levels of MG, DG and TG determined according to ASTM D6584. Contour plots of MG, DG, TG and conversion of the permeated FAME can be found in Figures 5-8, 5-9, 5-10 and 5-11 respectively.

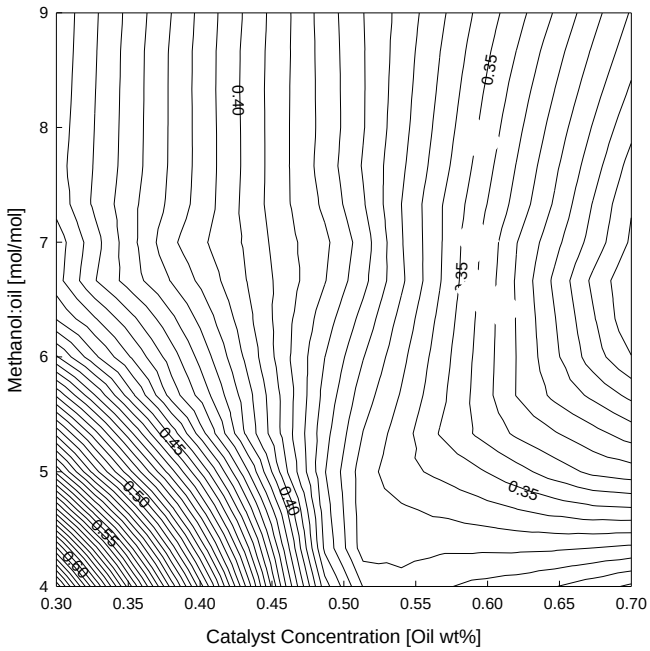


Figure 5-8: Contour plot of monoglyceride content [wt%] in permeated FAME

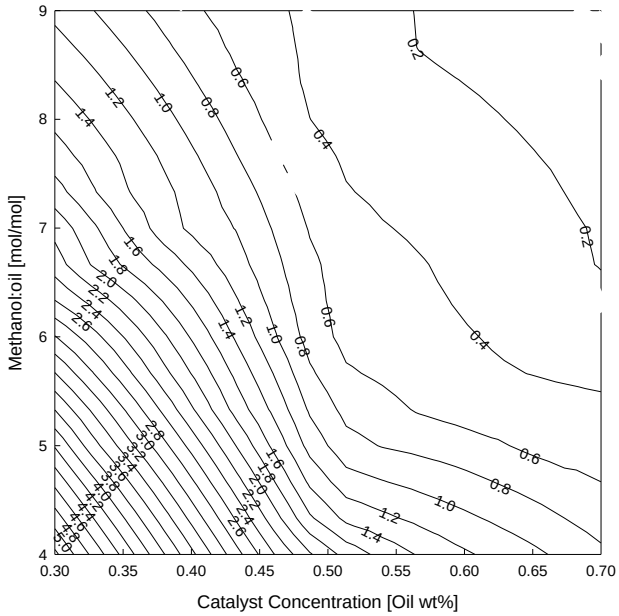


Figure 5-9: Contour plot of diglyceride content [wt%] in permeated FAME

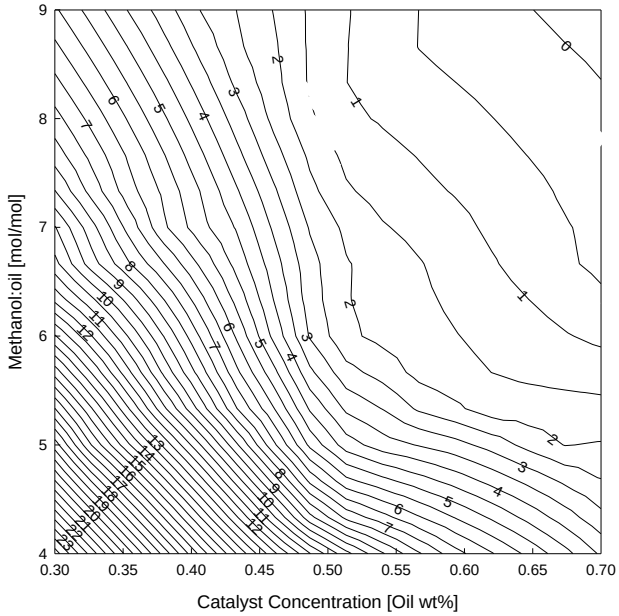


Figure 5-10: Contour plot of triglyceride content [wt%] in permeated FAME

At low methanol ratios, MG content is seen to decrease with increasing catalyst concentration. It is relatively constant at higher MeOH:Oil loadings. The MG content of all the FAME produced is less than 0.8 wt%, the limit set by EN 14214. DG content is seen to decrease with increasing catalyst concentration and increasing MeOH:Oil ratio for all methanol loadings. The runs at high MeOH:Oil and high catalyst concentration met the 0.2 wt% limit set by EN14214 while others did not. The unreacted TG content in the FAME is seen to decrease with increasing catalyst concentration and increasing MeOH:Oil ratio. The run at high MeOH:Oil and high catalyst concentration met the 0.2 wt% limit set by EN14214 while others did not.

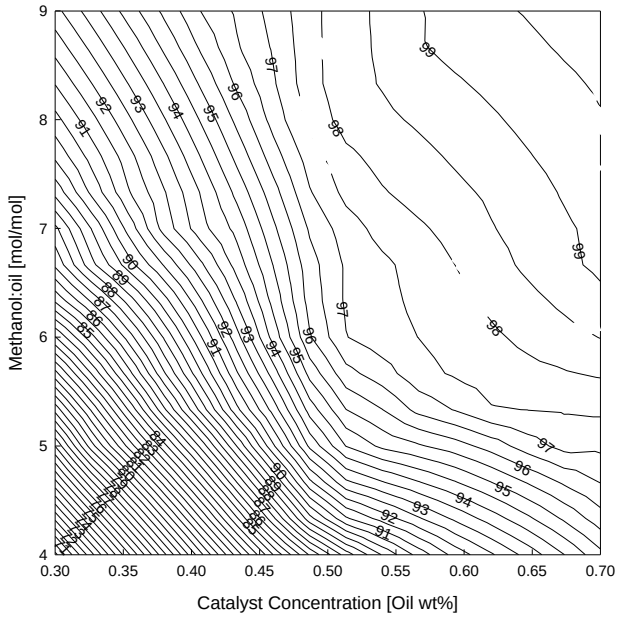


Figure 5-11: Contour plot of conversion [%] of permeated FAME

The conversion of saponifiable material in the oil is seen to increase with increasing catalyst concentration and MeOH:Oil ratio, which is consistent with general observations found in the literature.

3.4 The effect of MG, DG and TG

SG is known to be partially soluble in soybean oil (2300 ppm) [38]. The separation of SG was plotted against TG content (wt%) and can be found in Figure 5-13.

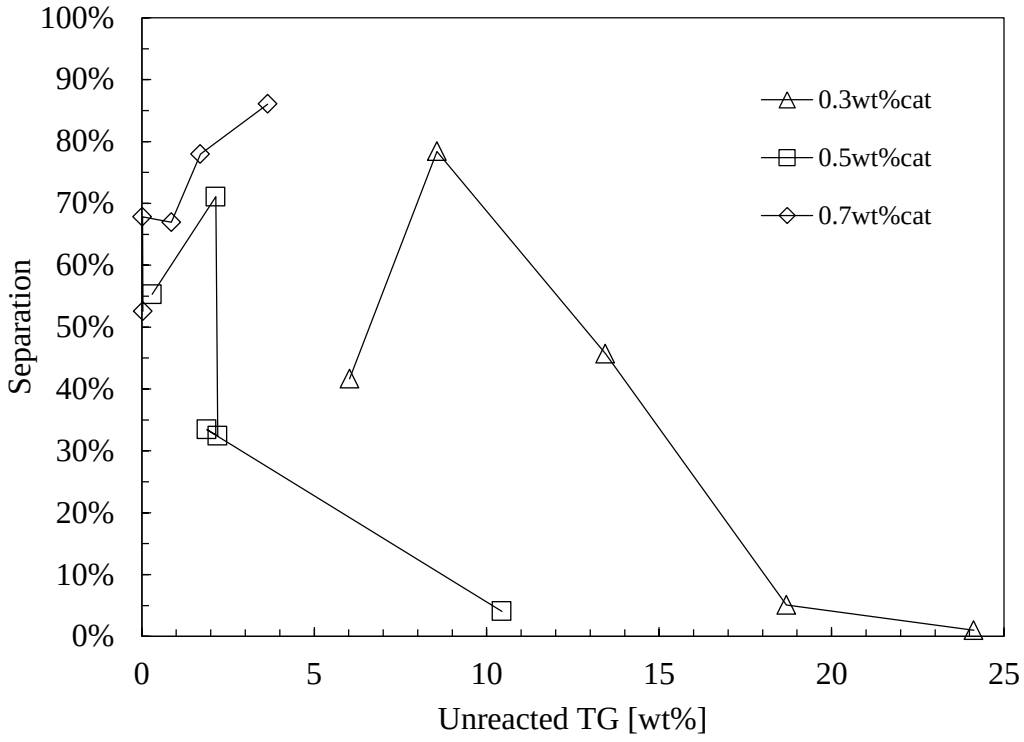


Figure 5-12: Separation vs unreacted TG [wt%] in the permeated FAME

The separation factor is seen to sharply increase, then decrease with increasing levels of TG. The increase in separation is due to excess methanol present in the phase which compatibilizes SG. This effect is discussed later. The decrease is attributed to the partial solubility of SG in soybean oil (2300 mg/kg). As the TG content in the FAME increases up to 24 wt%, the partial solubility of SG in the FAME phase also increases. This trend does not appear in the FAME samples produced from 0.7 wt% catalyst. These samples are low in TG and other factors dominate in explaining the solubility of SG in FAME under these conditions.

MG and DG can interact with the hydroxyl groups on SG and increase its solubility in FAME. The TG content of the permeated samples (wt%) was plotted against the number of hydroxyl groups (-OH mol%) in MG and DG calculated using Equation 2.

$$-\mathbf{OH}\ (\mathbf{mol}\%) = \frac{\mathbf{2} \times \frac{\mathbf{Mass_{MG}}}{\mathbf{MW_{MG}}} + \frac{\mathbf{Mass_{DG}}}{\mathbf{MW_{DG}}}}{\frac{\mathbf{Mass_{MG}}}{\mathbf{MW_{MG}}} + \frac{\mathbf{Mass_{DG}}}{\mathbf{MW_{DG}}} + \frac{\mathbf{Mass_{TG}}}{\mathbf{MW_{TG}}} + \frac{\mathbf{Mass_{BD}}}{\mathbf{MW_{BD}}}}. \quad (2)$$

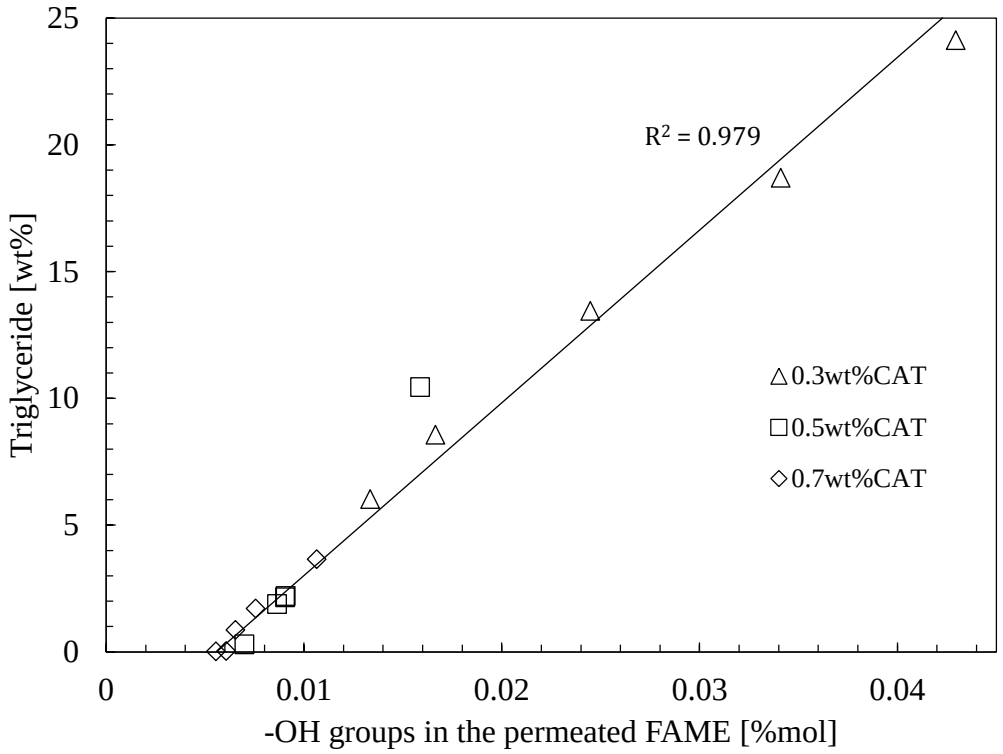


Figure 5-13: –OH groups [mol%] vs unreacted triglycerides [wt%] in the permeated FAME

The number of hydroxyl groups found in MG and DG is proportional to the fraction of unreacted TG. Differentating between the effects of unreacted TG and the presence of MG and DG in the FAME is difficult as both quantities are correlated in the finished FAME.

5.3.5 Effects of deprotonation

The lower pKa of stigmasterol glycoside was estimated at 12.21 [39]. During the transesterification reaction, the methoxide ions are predominantly found in the methanol/glycerol phase due to the polar nature of this phase. Unreacted TG is hydrophobic and lowers the catalyst concentration in the FAME phase. To study the effects of deprotonation on SG solubility in FAME; catalyst phase partitioning data [40] was used to estimate the pH in the reactive glycerol phase. The FAME samples produced using 0.7 wt% catalyst run were used to study the effects of deprotonation as they have high catalyst concentraion. Figure 5-14 shows the separation factor for FAME produced using 0.7wt% catalyst plotted against the estimated pH of the polar phase. This also represents the pH of the polar components (methanol/glycerol and other associated polar compounds) in the FAME phase.

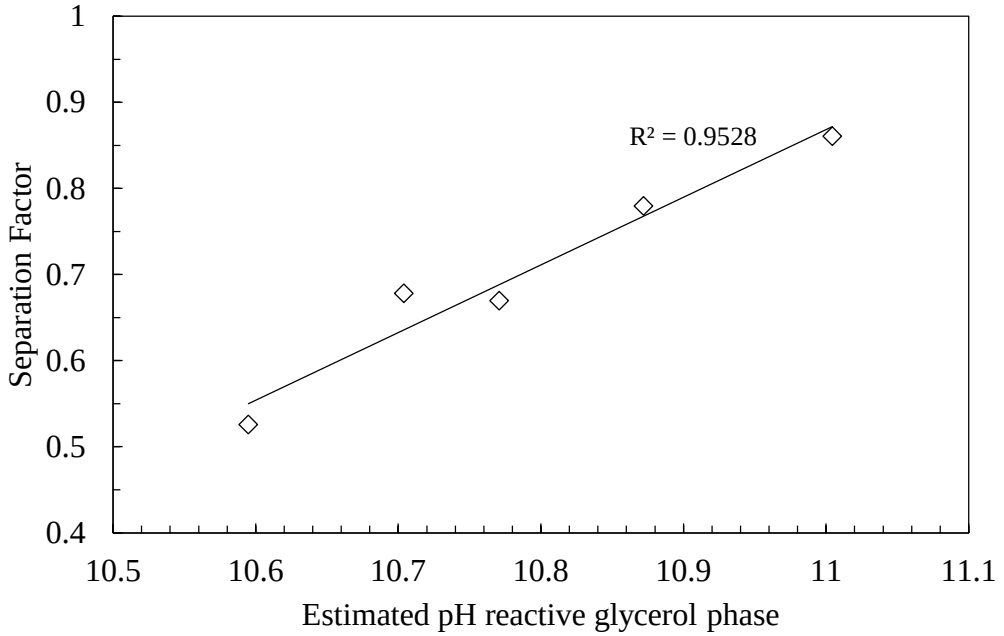


Figure 5-14: Separation factor vs estimated pH of reactive glycerol phase for FAME produced from 0.7wt%cat

As the catalyst concentration in the reactive glycerol phase increases, the methoxide further deprotonates the SG making it less soluble in the FAME phase.

5.3.6 Effects of excess methanol on solubility.

The value of the Hildebrand solubility parameter of a solvent mixture can be determined by averaging the values of the Hildebrand solubility parameter for individual solvents in a mixture on a volume basis. The solvent system of acetone/methanol (9:1, v/v) is used to elute SG in column chromatography and has a total Hildebrand solubility value of 20.90 $MPa^{0.5}$. A system of FAME/methanol with the same total Hildebrand value should exhibit similar solubility behaviour towards SG.

The Hildebrand solubility value of the reactive ester phase was modelled using methanol and catalyst phase partitioning data [40] and plotted in Figure 5-15 below.

The Hildebrand solubility parameter values of solvents can be found in Table 5-3. The presence of methanol in FAME increased the mixtures polar and hydrogen bonding components, and compatiblized SG in the phase.

Table 5-2: Hildebrand solubility parameter values of different solvents **[41]**

	δ_D [$MPa^{0.5}$]	δ_P [$MPa^{0.5}$]	δ_H [$MPa^{0.5}$]	δ_{Total} [$MPa^{0.5}$]
Methanol	15.1	12.3	22.3	29.6
Acetone	15.5	10.4	7.01	19.94
Biodiesel	15.03	3.69	8.92	17.86

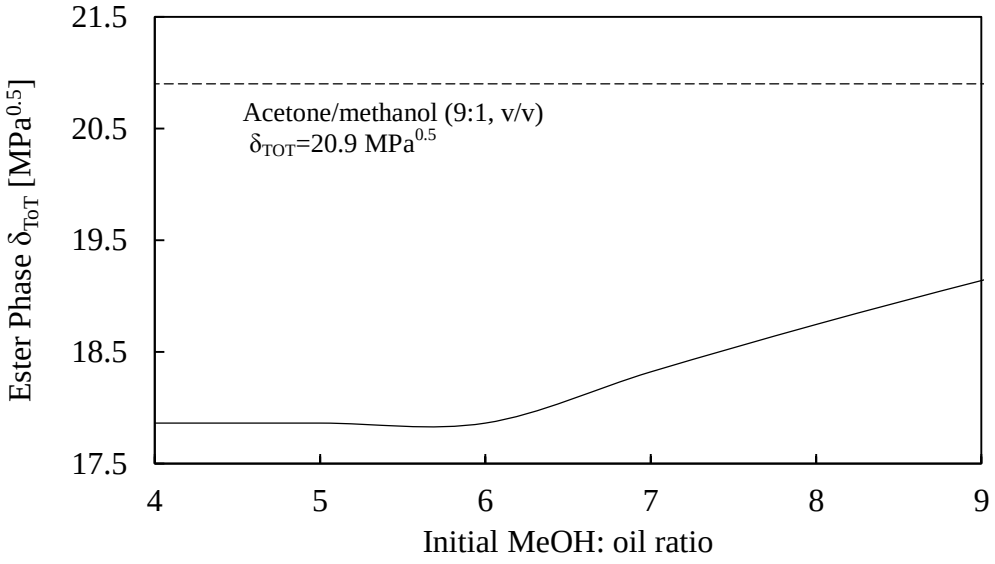


Figure 5-15: Estimated Hildebrand solubility value of final ester phase vs MeOH:Oil in initial reaction mixture

FAME made with an initial MeOH:Oil ratio higher than 6:1 will have excess methanol present in the ester phase, changing the solubility parameter value of the phase. As the fraction of methanol present in the ester phase increases, the mixture's solubility parameter increases towards that of chloroform/methanol (2:1, v/v) favoring the solubility of SG in FAME.

5.3.7 FAME produced at 0.7 wt% catalyst

To study the effects of ultrafiltration on FAME that is close to ASTM and EN standards, a summary table of minor components can be found in Table 5-2. The levels of MG, DG and TG in the FAME produced using 0.7wt% catalyst and MeOH:Oil ratios of 7:1 and 9:1 meet EN14214 standards. This was achieved using a single reaction step and ultrafiltration was able to reduce the SG levels by a factor of 68% and 52%, respectively. The lowest level of SG, 3.4ppm, was found in the permeate obtained for FAME produced using a MeOH:Oil ratio of 4:1. This corresponded to a separation factor of 86%.

Table 5-3: Summary of results for FAME produced using 0.7wt% catalyst

MeOH:Oil	Analysis in Permeate				[SG] retentate (ppm)	[SG] permeate (ppm)	SF (%)
	MG [wt%]	DG [wt%]	TG [wt%]	Conversion [%]			
4	0.391	0.804	3.65	95.2	24	3	87.5
5	0.317	0.454	1.796	97.5	34	7	79.4
6	0.309	0.286	0.859	98.5	32	10	68.7
7	0.302	0.119	0.018	99.6	17	5	70.6
9	0.329	0.124	0.033	99.5	24	11	54.2

3.8 Summary of results

A summary table of phenomena affecting sterol glycoside separation and solubility in FAME at the extreme corners of the analytical space listed in Table 5.1 can be found in Table 5-3.

Table 5-4: Summary table of the catalyst and MeOH:Oil levels affecting SG separation and the resulting solubility of SG in FAME

Catalyst level	Methanol level	Experimental coordinates	Separation	TG [wt%]	Reasons
+	-	0.7wt%cat, 4:1	86%	3.6	High ionization, low methanol in FAME. Limited solubility. SG rejected from FAME phase leading to high separation.
-	-	0.7 wt% cat, 4:1	0%	24	High TG levels solubilizes SG in FAME phase and transports them through the membrane
+	+	0.7 wt% cat, 9:1	52%	0.032	Excess MeOH increases solubility of SG in FAME. Lower TG decreases solubility in FAME. Leads to intermediate solubility.
-	+	0.3 wt% cat, 9:1	41%	6	Higher methanol in FAME, lower catalyst => less ionization. Intermediate TG. Net effect is intermediate solubility, higher solubility than + + case

5.4 Conclusion

To study the solubility of SG in a reactive biodiesel mixture, FAME was produced using a range of catalyst concentrations and MeOH:Oil ratios. The transesterification reaction lasted 60 min and the reactive mixture filtered through a 300 kDa MWCO ceramic membrane. The retained FAME mixture was separated from the glycerol phase using gravity settling and both FAME streams neutralized with HCl. Excess methanol was removed using a rotary evaporator. None of the FAME produced in this study was water washed in order to conserve the SG concentration in the reactive phases. SG was extracted from FAME by decompatibilization using n-dodecane at low temperatures. Non-polar compounds in the precipitates were isolated from polar compounds using a Folch liquid-liquid extraction. Neutral compounds were then further washed in a small volume of n-dodecane. The SG concentration from both streams was analyzed using GC-FID. The separation factor of SG in the FAME was calculated using these values. The FAME produced was also characterized for MG, DG and TG in accordance with ASTM D6584.

SG separation by a 300 MWCO kDa decreased with increasing unreacted TG content. SG is partially soluble in soybean oil (2300 ppm), and unreacted oil present in the FAME will solubilize SG in the ester phase. The compatibility of SG in poorly converted FAME mixtures allows SG to pass through the membrane.

Deprotonation effects were observed to influence SG levels in the permeated FAME. The SG levels in the permeated FAME decreased with increasing catalyst concentration in the reactive glycerol phase. As the catalyst concentration increased, the methoxide further deprotonated the hydroxyl groups in SG and made it less soluble in FAME, increasing the separation factor.

Excess methanol was found to increase SG solubility in FAME. This was evaluated in relation to the Hildebrand solubility parameter of the reactive FAME mixture. As the volume fraction of methanol in the FAME phase increased, the polar and hydrogen bonding components of the FAME mixture increased and partially solubilized SG. Excess methanol increases the compatibility SG within the mixture, leading to lower separation.

It was observed that the highest separation for SG (86%) was obtained at the highest deprotonation levels where the reaction conditions were 0.7 wt% and 4:1 MeOH:Oil ratio. The separation was the lowest (0%) at 0.3 wt% and 4:1 MeOH:Oil ratio where unreacted triglycerides and intermediates such as MG and DG were found to solubilize SG in the mixture.

The use of a membrane separation process during the transesterification reaction was highly beneficial in separating SG from biodiesel. The lowest levels of SG, 3.4ppm, were found in the permeate obtained for FAME produced using a MeOH:Oil ratio of 4:1. A further second reaction could be used to meet ASTM and EN levels for MG, DG and TG. The product of this second reaction would contain very low level of SG without the need for distillation, cold soak filtration, absorbents, enzymes or water washing.

Chapter 6

Conclusions and Recommendations

6. Conclusions

Chapters 1-3

The decline in crude oil reserves and potential impacts of global warming has made biodiesel an economically viable alternative. Biodiesel is a renewable and environmentally friendlier alternative to conventional diesel fuel, with many added benefits. Although biodiesel does have some attractive advantages, certain disadvantages are hindering its widespread acceptance and adoptions. Currently, biodiesel has poor low temperature operability and stability caused by high levels of minor components such as MG, DG and SG. Currently, only Europe has implemented standards which place restrictions on partially reacted glycerides, but no such standards exist for sterol glycoside levels.

SG concentration in crude soybean oil has been reported to be as high as 2300ppm, and 500ppm and 300ppm in corn and safflower oil, respectively. In its acylated form, ASG is completely soluble in triglycerides and is found in biodiesel raw materials. During the transesterification reaction, ASG is deacylated to SG which is insoluble in biodiesel. The compounds crystalize out of solution causing fuel haze. In high enough concentration, they can form cloud-like agglomerations which have been shown to act as crystal seeds for other compounds such as MGs and DGs. They are currently removed either by distillation, or cold filtration of biodiesel; both of which increase the cost of production.

Chapter 4

A new extraction and detection method was developed to analyze SG in FAME and biodiesel samples. SG were decompatibilized from the fuel matrix by diluting FAME in n-dodecane, a low-aromatic aliphatic hydrocarbon. The B50 solutions were cold soaked for 16hours at -8^{o}C and centrifuged to isolate precipitates. The polar compounds were removed using a Folch liquid-liquid extraction and the non-polar compounds were washed with a small amount of n-dodecane. The solids were then sylilated with 100µL of MSTFA and 100µL of a tricaprin standard was added. This solution was diluted in 4mL of anhydrous heptane before being analyzed by GC-FID using the same operating conditions outlined in ASTM D6584.

To determine the relative retention time of SG, they were isolated from crude non-degummed soybean using column chromatography. Using the operating conditions outlined by ASTM D6584, the tricaprin was detected at 21.5mins and SG at 26-28mins for a RRT = 1.21-1.3. A calibration curve was prepared from these SG. A first order multiple peak integration showed an excellent correlation (R^2=99). The detection limit was measured at 2 ppm and can be reduced by changing sample size and/or the final heptane dilution rate prior to GC injection.

This new extraction method was used to determine the SG concentration in biodiesel samples spiked with 38ppm of SG and showed recovery rates of 99% to 100%. The non-reliance on a manufactured product such as a filtration device or SPE extractant, low detection limit and high recovery of the new analytical method should form the basis of an ASTM standard for SG analysis in biodiesel.

Chapter 5

FAME was produced from degummed soybean oil using 0.3wt%, 0.5wt% and 0.7wt% catalyst and 4:1, 5:1, 6:1, 7:1 and 9:1 initial MeOH:Oil (mol/mol). The reactive mixture was filtered through a 300kDa MWCO membrane until 100mL of permeate was collected. The retentate was separated from the reactive glycerol phase by gravity settling. Both the retentate and permeate streams were neutralized with concentrated HCl and distilled to remove excess methanol. The SG was extracted using the method described in Chapter 4. These solids were sylilated with 100μL MSTFA and 100μL of 8mg/mL tricaprin standard was added. The sample was diluted in 4mL of anhydrous heptane and 1μL sample injection was used for GC-FID analysis. The tricaprin standard was detected at 21.5mins and SG at 26-28mins with a RRT=1.21-1.3. A calibration curve was produced using 98%+ purity sterol glycoside standard and a multiple peak linear fit was found to work best. The samples were also characterized for MG, DG and TG using the method described in ASTM D6584.

Separation decreased when unreacted TG levels increased. SG is partially soluble in soybean oil (2300ppm) and high levels of unreacted TG solubilize SG in the FAME phase. Catalyst concentration also had effects on SG levels in the permeate stream. As the catalyst concentration increases, the methoxide further deprotonates the hydroxyl groups in SG and make it more soluble in the polar glycerol phase. This effect was most noticeable in highly converted FAME, thereby reducing the effect of unreacted TG on the solubility. Excess methanol was found to increase SG solubility in FAME. The methanol fraction in the FAME phase increases the mixture's polar and hydrogen-bonding Hildebrand values towards those of chloroform/methanol (2:1, v/v), a solvent system known to solubilize SG.

General recommendations

Very little information on sterol glycosides in triglyceride oils and biodiesel is available in the literature. Studying the solubility of SGs in a reactive FAME mixture is inherently difficult given the complexity of triglyceride mixtures, which contain many oil-soluble compounds that may interact with ASGs or SGs during the reaction.

ASG concentration could potentially be measured by determining its RRT. The decrease in ASG, and increase of SG, during the transesterification reaction could be measured under different reaction conditions. The effects of the n-dodecane on ASG extraction reproducibility should be studied, as ASG is known to be soluble in oil.

Other types of oils with different SG content could be used during the transesterification reaction. With slight modification to the GC operating conditions, another method could be developed to speciate SG, and differentiate between biodiesel sources.

Different effects, such as deprotonation of SG, could be utilized to minimize SG concentration in the filtered FAME. A second transesterification reaction could then be performed to meet EN 14214 standards. This would result in EN-grade biodiesel with very low SG concentration.

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Appendix

Sample Preparation

1. Weigh 13g of FAME (15mL) into a 50mL PP centrifuge tube
2. Weigh 13g of n-dodecane (NBP 215-217oC) into the same tube
3. Add 10µL of 37% HCl
4. Close top and vortex for 2mins to maximize contact between the FAME and acid
5. Place samples in ethylene glycol/water bath at -8oC for 16 hours
6. Centrifuge samples at 6000rpm, -8°C for 45mins in temperature controlled centrifuge
7. Decant clear liquid without disturbing the pellet
8. Dissolve the isolated pellet in 10mL of chloroform/methanol (2:1, v/v)
9. Add 2mL of 0.9wt% NaCl distilled water to the dissolved solids
10. Vortex for 5mins to ensure phase contact
11. Let liquid-liquid extraction system settle for 15mins
12. Remove top aqueous phase by suction and check that pH<6
13. Dry bottom organic phase using nitrogen gas directly in the tube. Water droplets should be seen to form towards the end. These should be removed as much as possible.
14. Add 5mL of n-dodecane to the dried solids and shake
15. Centrifuge samples at 2000rpm for 45mins in the variable angle centrifuge
16. Pipette off the n-dodecane leaving behind a clearly defined pellet
17. Add 1mL of chloroform/methanol (2:1, v/v) to dissolve solids
18. Transfer dissolved solids to glass vessels and rinse the tube 3x with chloroform/methanol
19. Dry dissolved solids using nitrogen
20. Add 100µL of tricaprin standard in pyridine (8mg/mL)
21. Add 100µL of MSTFA and let react for 45mins at room temperature
22. Dilute sample in 4mL of anhydrous heptane
23. Transfer about 1.5mL to a GC autosampler vial
24. Analyze by GC, measure area from RRT = 1.21-1.3 (26-28mins)