OPTIMIZATION OF *IN-SITU* TRANSESTERIFICATION PROCESS FOR SOYBEAN AND SPENT COFFEE GROUNDS BIODIESEL

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การเพิ่มประสิทธิภาพกระบวนการ อินซิตู ทรานส์เอสเตอร์ริฟิเคชั่น สำหรับผลิตไบโอดีเซลจากถั่วเหลืองและกากกาแฟ

นายณัฐพงศ์ ตันติวิวัฒนพันธ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Thesis Title	OPTIMIZATION OF *IN-SITU* TRANSESTERIFICATION PROCESS FOR SOYBEAN AND SPENT COFFEE GROUNDS BIODIESEL
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Field of Study	Environmental Management
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ณัฐพงศ์ ตันติวิวัฒนพันธ์ : การเพิ่มประสิทธิภาพกระบวนการ อินซิตู ทรานส์เอสเตอร์ริฟิเคชั่นสำหรับผลิตไบโอดีเซลจากถั่วเหลืองและกากกาแฟ (OPTIMIZATION OF *IN-SITU* TRANSESTERIFICATION PROCESS FOR SOYBEAN AND SPENT COFFEE GROUNDS BIODIESEL) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. จันทรา ทองคำเภา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร. Dennis P. Wiesenborn, 194 หน้า.

ถั่วเหลืองและกากกาแฟได้ถูกนำมาใช้สำหรับผลิตไบโอดีเซลผ่านกระบวนการ “อินซิตู ทรานส์เอสเตอร์ริฟิเคชั่น” (*In-situ* transesterification; *in-situ* TE) กระบวนการนี้สามารถลดความซับซ้อนในการผลิตไบโอดีเซลโดยจะควบรวมขั้นตอนการสกัดน้ำมันและขั้นตอนการสังเคราะห์ไบโอดีเซลเข้าด้วยกัน ตัวแปรที่เกี่ยวข้องกับการสกัดและการสังเคราะห์ไบโอดีเซลได้ถูกนำมาประเมินผลกระทบต่อประสิทธิภาพในการผลิตไบโอดีเซล และนำไปหาสภาวะที่เหมาะสมของกระบวนการ *In-situ* TE ผลที่ได้ออกมาแสดงให้เห็นว่า การเลือกใช้สารละลายแอลกอฮอล์ที่มีความเป็นขั้วต่ำสามารถเพิ่มประสิทธิภาพในการผลิตไบโอดีเซลและลดการใช้ปริมาณแอลกอฮอล์และสารเร่งปฏิกิริยา รวมถึงสามารถผลิตไบโอดีเซลโดยใช้อุณหภูมิที่ต่ำลงและในเวลาที่สั้นลง การล้างกากกาแฟโดยใช้เมทานอลสามารถลดค่าความเป็นกรดในกากกาแฟได้อย่างมีประสิทธิภาพและปรับสภาวะให้เหมาะสมต่อกระบวนการ *In-situ* TE การเพิ่มอุณหภูมิในการผลิตสามารถแก้ไขปัญหาความหยาบของขนาดกากกาแฟที่ใช้ในการผลิตไบโอดีเซล โดยประสิทธิภาพการผลิตไบโอดีเซลจากกากกาแฟอยู่ที่ 80% ภายในเวลา 3 ชั่วโมง โดยใช้กากกาแฟ 4 กิโลกรัม ซึ่งไบโอดีเซลที่ได้มีค่าต้านทานปฏิกิริยาออกซิเดชันสูงมากเนื่องจากสารต้านอนุมูลอิสระตามธรรมชาติของกากกาแฟที่ถูกสกัดออกมาพร้อมกันกับไบโอดีเซล การประเมินวัฏจักรชีวิตของกระบวนการผลิตไบโอดีเซลจากกากกาแฟโดยใช้พื้นที่ภายในโรงงานผลิตกาแฟสำเร็จรูปผ่านกระบวนการ *In-situ* TE แสดงให้เห็นว่ามีการลดลงของผลกระทบที่มีต่ออวัยวะการหายใจและการใช้พื้นที่เมื่อเปรียบเทียบกับการผลิตโดยกระบวนการผลิตไบโอดีเซลที่ใช้กันในปัจจุบัน เนื่องจากกระบวนการในงานวิจัยนี้ไม่มีการใช้สารละลายเฮกเซนและไม่จำเป็นต้องมีระบบการขนส่ง แต่ทว่ากระบวนการนี้ใช้พลังงานสูงมากในขั้นตอนการกลั่นเอาเมทานอลกลับมาใช้ใหม่ ซึ่งส่งผลกระทบต่อภาพรวมของสุขภาพ คุณภาพของระบบนิเวศ การเปลี่ยนแปลงของสภาวะอากาศ และทรัพยากรสิ่งแวดล้อม สุดท้ายการผลิตไบโอดีเซลโดยวิธีที่พัฒนาขึ้นจะใช้พลังงานน้อยกว่ากระบวนการที่ใช้กัน ณ ปัจจุบัน ต่อเมื่อมีระยะทางในการขนส่ง >180 กม. ที่อัตราการสิ้นเปลืองน้ำมัน 7 กม./ล. สำหรับการลดการใช้พลังงานและผลกระทบต่อสิ่งแวดล้อมของกระบวนการในอนาคต สามารถทำได้โดยการนำเมทานอลและตัวเร่งปฏิกิริยากลับมาใช้ใหม่โดยไม่ต้องผ่านกระบวนการกลั่นเมทานอล หรือเปลี่ยนไปใช้การสกัดแบบต่อเนื่อง เพื่อลดปริมาณเมทานอลและตัวเร่งปฏิกิริยาที่ใช้ในกระบวนการผลิต

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต ....
ปีการศึกษา 2559 ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
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NATTAPONG TUNTIWIWATTANAPUN: OPTIMIZATION OF *IN-SITU* TRANSESTERIFICATION PROCESS FOR SOYBEAN AND SPENT COFFEE GROUNDS BIODIESEL. ADVISOR: ASST. PROF. CHANTRA TONGCUMPOU, Ph.D., CO-ADVISOR: PROF. DENNIS P. WIESENBORN, Ph.D., 194 pp.

Soybean and spent coffee ground (SCG) were applied as biodiesel feedstock in the reactive extraction process named *in-situ* transesterification (*in-situ* TE). This process reduced the complexity of biodiesel production by combining vegetable oil extraction and biodiesel synthesis into a single step. Several parameters were evaluated for the effect on biodiesel yield. The optimal process conditions using different alcohol systems showed that the stronger hydrophobicity of alcohol system provided the higher biodiesel yield. It also reduced the alcohol and catalyst loading, lower the reaction temperature and shorten the operation time. Simply washing SCG by methanol could reduce acidify of SCG. Elevating temperature could compensate the coarse particle size of SCG. The kinetic results showed that 80% of biodiesel yield could be obtained within 3 h using 4 kg SCG loading. SCG biodiesel produced from *in-situ* TE had high oxidative stability index due to the co-extraction of antioxidant. LCA results showed that producing SCG biodiesel at an on-site instant coffee process via *in-situ* TE had lower impacts at industrial level in terms of respiratory organ and land occupation than those of conventional approach because of the absence of n-hexane and transportation. However, it consumed much more energy during methanol recovery step, resulting in more impacts in the overall of health, ecosystem quality, climate change and resource. The sensitivity analysis suggested that producing biodiesel at an on-site was more favorable in term of energy usage once the transportation distance was >180 km with 7 km/L of fuel consumption rate. In the future work to improve energy usage and environmental impact of our process, we could directly reuse catalyzed methanol in the process, or apply countercurrent extraction technique which could reduce the amount of methanol and catalyst in the process.

Field of Study:	Environmental Management	Student's Signature	....
Academic Year:	2016	Advisor's Signature	....
		Co-Advisor's Signature	....

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CHAPTER 1

GENERAL INTRODUCTION

1.1. Introduction

Encouraging by volatile petroleum oil price and poor environmental quality, the use of vegetable oil as fuel in a diesel engine has been extensively study in the past decades (Yusuf et al., 2011). In comparison with diesel fuel, vegetable oils have a superior ignition quality (cetane number), and the exhausted gas is lower in sulfur, carbon dioxide and aromatic polycyclics hydrocarbon (Knothe et al., 2005; Knothe and Dunn, 2005). High viscosity of vegetable oil, however, is its limitation to be directly used in the diesel engine. Several methods have been developed to reduce a high viscosity of vegetable oil, including dilution with petroleum diesel (Pramanik, 2003), microemulsification (Dantas et al., 2000; Ploysrimongkol and Tongcumpou, 2009; Nguyen et al., 2010) and thermal cracking by pyrolysis (Srivastava and Prasad, 2000). Transesterification (TE) is the most applied chemical reaction process by converting triglyceride (majority of vegetable oil’s component) to fatty acid alkyl ester (i.e. biodiesel), commonly using an alkali or acid-catalyzed methanol (Knothe et al., 2005; Walker, 2004; Yusuf et al., 2011). In addition, biodiesel production can add value of the industry by using its waste as non-edible biodiesel feedstock (i.e. waste-to-energy concept) such as waste cooking oil (WCO) from food industry (Wan Omar et al., 2009), activated sludge from wastewater treatment plant (Gunawan et al., 2014; Choi et al., 2014) and spent coffee grounds (SCG) from coffee industry (Vardon et al., 2013).

Rural communities could potentially produce and use their own biodiesel from locally-grow oil crop. However, there were several limitations those require the innovative technology to accomplish. Biodiesel production is generally comprised of complex processes involving multiple seed pretreatment steps, vegetable oil extraction via mechanical pressing and/or solvent, vegetable oil purification, biodiesel synthesis and refinery. Among all of them, solvent oil extraction process commonly using n-hexane was the major concern since it has been categorized as a hazardous air pollutant (Clean Air Act, 1990). This extraction process requires advance systems and high-skilled workers to prevent the leaking of hexane which is easily volatile and flammable. Several accidents relating to vegetable oil hexane extraction have been reported. The most serious case occurred in Denmark in 1980, where hexane was released at a soybean oil extraction plant, resulting in 27 casualties (Landucci et al., 2011). Moreover, such a process will be economic feasible at a production scale of >2,400 tons oilseed per day (Hass et al., 2004). The oilseed supply from a single rural area might not be sufficient for this kind of process. Transportation systems are additionally required for oilseed and biodiesel transportation. Hence, a smaller biodiesel production process with an absence of n-hexane will be more feasible and attractive.

A reactive extraction process named *in-situ* transesterification (*in-situ* TE) might be a suitable alternative process for use in a small on-site plant. Catalyzed alcohol is used as a biodiesel reactant and as extraction solvent. The main concept of this approach is to convert the triglyceride within the oilseed to biodiesel and glycerol and extract them by catalyzed alcohol, simultaneously. Therefore, it can reduce the size and complexity of the biodiesel production system (Tuntiwiwattanapun et al., 2016; Haagenson and Wiesenborn, 2011). However, a large amount of methanol was used in this process, which is considered as the major weakling. Tremendous energy is required during a methanol recovery step, resulting in low energy efficiency and high environmental impacts (Nazir et al., 2012). To make such a process more feasible, the reduction of methanol requirement is very important.

In our point of views, the successful element of *in-situ* TE process might be improving the interaction between triglyceride and catalyzed alcohol. Methanol is commonly used in a conventional biodiesel production since it is cheaper and its reaction rate of TE is faster than the other alcohols. However, in case of *in-situ* TE, there is a limitation of using methanol because most of the oil is entrapped inside the oilseed which is difficult to access by poor oil solubility alcohol such as methanol, resulting in low performance of *in-situ* TE process.

In this study, four main approaches were introduced to improve the performance of the *in-situ* TE process, which were (1) using more hydrophobic alcohol than methanol (ethanol and isopropanol), (2) adding co-solvent (acetone), (3) reducing water content in both oilseed and catalyzed alcohol and (4) optimizing extraction conditions such as process temperature, time, alcohol loading and particle size of the oilseed. Soybean and spent coffee grounds (SCG) were selected as the representative of biodiesel feedstock for remote area and industrial waste, respectively. A design of experiment (DOE), named Taguchi orthogonal array method (Taguchi) and central composite rotatable design (CCRD), were applied for screening the crucial factors and optimizing the *in-situ* TE conditions. Then, the developed *in-situ* TE process was scaled up and evaluated the energy usage and environmental impacts based on life cycle assessment (LCA) method.

1.2. Objectives

1. Screening the crucial factors on biodiesel yield of the *in-situ* TE process.
2. Optimizing the process conditions of *in-situ* TE and scaling up the developed process.
3. Evaluating and comparing energy usage and environmental impacts of *in-situ* TE and conventional biodiesel process.

1.3. Hypothesis

1. Alcohol systems which have a higher oil solubility will provide a higher biodiesel yield as well as reduce operation time, temperature and alcohol loading.
2. Particle size reduction and removal of water and free fatty acid in oilseed will increase the biodiesel yield.
3. Elevating temperature will increase biodiesel yield and reduce the alcohol loading in the process.
4. Applying *in-situ* TE as an on-site biodiesel production will lower energy usage and environmental impacts compared to conventional seed-to-biodiesel process.

1.4. Study Framework

The study framework of this dissertation were separated into two sections based on biodiesel feedstock which were soybean and SCG, and each section had two parts (Figure 1.1). Soybean was selected as a feedstock in Section I since soybean provides a good oil quality (i.e. no need for vegetable oil purification), thus it was suitable for the fundamental study on *in-situ* TE. Part 1 was to identify the quantitatively effect of 8 individual parameters related to performance of the soybean *in-situ* TE process. Part 2 aimed to optimize the conditions of the selected parameters which yielded the most effective on the performance of the *in-situ* TE (i.e. biodiesel yield), which were identified in Part 1. The results obtained from the soybean study had been applied to develop the *in-situ* TE process for producing SCG biodiesel in Section II. Due to the poor oil quality of SCG, the pretreatment process (deacidifcation) had to be developed. The optimized conditions were evaluated the kinematic parameters, and then scaled-up to 4 kg SCG loading per batch. Lastly, the final part of this dissertation was to evaluate the energy usage and environmental impacts of developed *in-situ* TE from Part 3 according to LCA method.

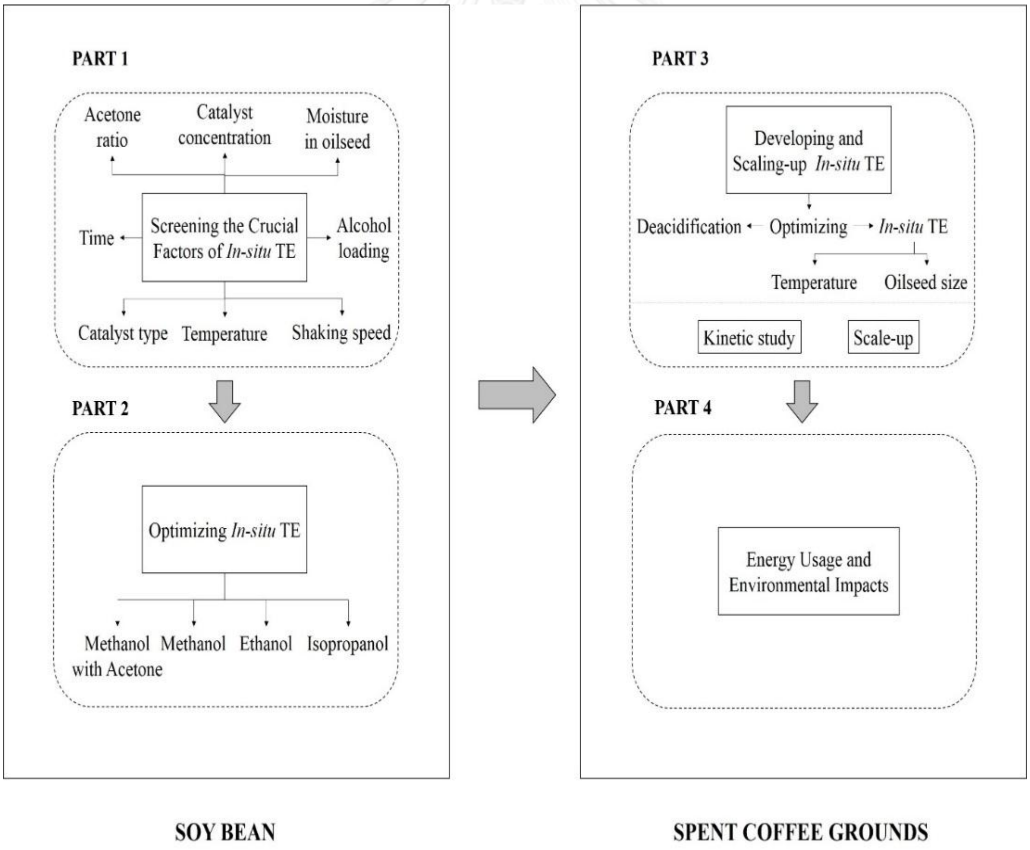


Figure 1.1 Schematic flow chart of the framework of this study.

1.5. Structure of Dissertation

The dissertation was divided into 8 Chapters. Chapter 1: *"General Introduction"* described background and justification of why an *in-situ* TE was introduced and developed in this research work. The objectives and hypothesis of the study, which were the direction for the study framework and experimental design, were included in this chapter. Chapter 2: *"Literature Review"* introduced all aspects related to this work to readers including biodiesel, soybean, SCG, *in-situ* TE, LCA, as well as statistical tool used for the experimental design. In addition, all previous works related for each aspects were discussed. Chapter 3: *"Methodology"* described all details of materials, and procedures used in this study both for the experiments and data analysis.

The results of the study in both sections were separated into four chapters (chapter 4 – 7) according to the experimental designs and data analysis. Chapter 4: *"Screening Crucial Factors of In-Situ TE"* expressed the results obtained from the experiments varying parameters that affected on triglyceride extraction and biodiesel yield using Taguchi method for the experimental design. Chapter 5: *"Optimizing In-Situ TE for Producing Soybean Biodiesel"* explained the further steps using the results from chapter 4 to determine the optimum conditions of the soybean biodiesel production from four different alcohol systems (neat and mixture), alcohol loading, and catalyst concentration using CCRD for design of the experiment.

Chapter 6: *"In-Situ TE for Biodiesel Production Using SCG"* describes all experiments related to SCG which includes characterization of SCG, pretreatment process by deacidification and determination of the optimum conditions of the *in-situ* TE for producing SCG biodiesel. In addition, the results of biodiesel yield obtained from the scaling up for SCG biodiesel production as a pilot scale and the quality of the biodiesel were reported in this chapter.

Chapter 7: *"Energy Usage and Environmental Assessment of SCG Biodiesel Production"* was the last chapter demonstrating the result of the study. This chapter focused on the comparison of two scenarios (conventional and on-site in-situ TE processes) of the SCG biodiesel production in terms of the energy usage and environmental impacts of the production process according to LCA method. The details of this chapter included goal and scope of the analysis, how to obtain the data as well as life cycle inventory and life cycle impact assessment.

Chapter 8: *"Conclusion and Future Work"* summarized all key findings of this study. Several aspects that have not yet carried out within this study, but would make the research work more valuable and be able for the real application were described in the recommendations for future work.

CHAPTER 2

LITERATURE REVIEW

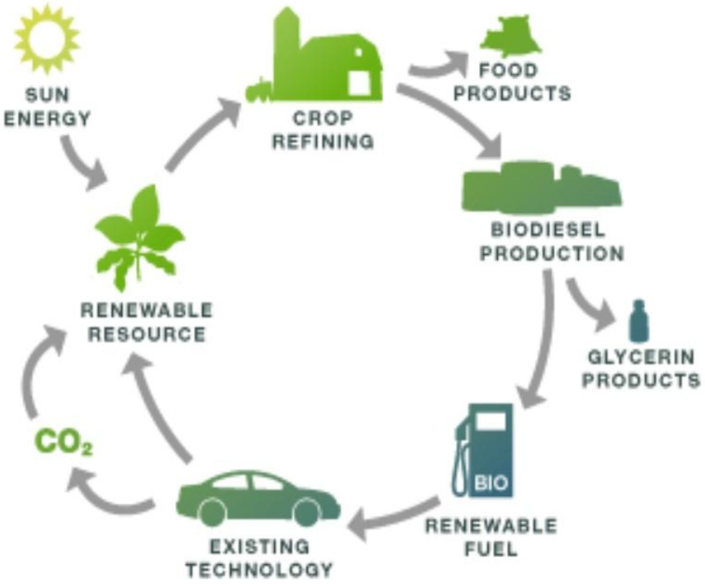
2.1. Biodiesel

Biodiesel is a mono-alkyl ester of long chain fatty acid which is a derivative product of vegetable oil or animal fat. It can be blended with petroleum diesel or directly used as alternative liquid fuel in conventional diesel engines (American Society for Testing and Materials, 2009). In addition to certify as biodiesel, the fatty acids alkyl ester must pass the biodiesel standard testing such as ASTM D6751 used in USA and DOEB B100 used in Thailand as summarized in Table 2.1.

The main concept of biodiesel production might be well explained as *"From Farm to Fuel"* presented in Figure 2.1. Vegetable oil is extracted from oilseed crops, which consume carbon dioxide (CO_2) emitted from car exhaustion as carbon source and sun light as energy source. Following this concept, several works consider biodiesel as a zero carbon emission or carbon neutral. However, it should be noted that beside of the biodiesel production and use, deforestation and fertilizer production during cultivation are accounted for the majority of CO_2 emission. Furthermore, the food shortage and land used change has become serious debates for promoting biodiesel, which currently uses edible feedstock for biodiesel production. In order to develop the biodiesel production in a sustainable way, the advantages and disadvantages of biodiesel should be considered, which were described in the subsequence sections.

Table 2.1 ASTM D6751 and DOEB B100 standard

Property	Unit	ASTM D6751	DOEB B100
Flash point (closed cup)	˚C	130 min	120 min
Water and sediment	% v/v	0.05 max	0.05 max
Kinematic viscosity, 40°C	mm^2/s	1.9-6.0	3.5-5.0
Sulfated ash	% w/w	0.02 max	0.02 max
Sulfur	% w/w	0.05 max	0.001 max
Copper strip corrosion		No.3 max	Class 1
Cetane number		47 min	51 min
Cloud point	˚C	Report	Report
Carbon residue (100 % sample)	% w/w	0.05 max	-
Acid number	mg KOH/g	0.50 max	0.50 max
Free glycerin	% w/w	0.02 max	0.02 max
Total glycerin	% w/w	0.24 max	0.25 max
Phosphorus content	% w/w	0.001 max	0.001 max
Oxidative stability index	h	6 min	10 min



http://farmwars.info/wp-content/uploads/2010/07/farm2fuel.gif

Figure 2.1 Biodiesel concept: from farm to fuel

2.1.1. Advantages of Biodiesel

2.1.1.1. Availability, renewability and applicability

The biodiesel feedstocks are commonly available in an agriculture section which is considered as renewable source such as soybean, palm, canola and jatropha. Biodiesel can be directly used in a commercial diesel engine as neat (B100) or blended with petroleum diesel fuel. In Thai market, the most common is a mix of 2, 3 and 5% biodiesel with petroleum diesel (B2, B3 and B5).

2.1.1.2. Strengthen energy security

Thailand is considered as a net energy importer since we imported more than 50% of energy in 2013, especially crude oil accounting for 81% of the imported energy (Alternative Energy and Efficiency Information Center, 2013). Diversifying and growing of renewable source could offer viable option to improve Thailand energy security. In 2021, the renewable energy will be responsible for 25% of energy consumption of Thailand. The biodiesel product will be double from 2.8 million L per day in 2013 to 5.97 million L per day in 2021 targeted year (Twarath, 2013).

2.1.1.3. Biodegradability

Increase in environmental concerns, the biodegradable fuel such as biodiesel is growing interest. The degradation rate of biodiesel is four time faster than that of petroleum diesel since the oxygen content in biodiesel structure (Demirbas, 2008). In natural environment condition, there is a report that 98% of rapeseed biodiesel is degraded within 21-day period, while only 60 % of pure fossil diesel fuel is decomposed (Yusuf et al., 2011).

2.1.1.4. Lubricity enhancement

The low sulfur content is a requirement for diesel fuel standard. In 1993, diesel fuel which was sold in USA, has sulfur content more than 5,000 ppm. The sulfur content in diesel fuel must be 15 ppm or less based on standard in 2006. A hydrotreating is generally used to reduce sulfur content in petroleum industry. However, it must scarify with the poor lubricant property which can damage the pistol and injection system in diesel engine. Scuffing load ball on cylinder lubricity evaluator and high frequency reciprocating rig are widely used as lubricity standard in engine manufacturer (Schumacher, 2005). Research demonstrates that high oxygen and nitrogen content in biodiesel which naturally has low sulfur makes biodiesel has good lubricant properties. Blending 1-2 % of biodiesel with low sulfur petroleum diesel provides sufficient lubrication for diesel engines (Beach and Schumacher, 2004).

2.1.1.5. Improving engine combustion performance

Cetane number (CN) is associated with the ignition quality of diesel fuel. It is commonly used as a diesel fuel quality standard (Knothe et al., 2005). The ASTM standard of diesel fuels states that the CN of diesel and biodiesel are 40 and 47, respectively (Yusuf et al., 2011). Generally, CN of biodiesel is higher than that of petroleum diesel, since biodiesel has the longer fatty acid carbon chains and the more saturated molecule (Bala, 2005).

2.1.1.6. Non-fuel application

In spite of fuel application, biodiesel has been considered as the industrial solvent for cleaning and degreasing (Wides, 2002; Hu et al., 2004) as well as cleaning up of oil spill (Miller and Mudge, 1997). The application of biodiesel as environmental friendly solvent for vegetable oil extraction should be interesting for future work.

2.1.1.7. Promoting rural economic and welfare

Since the biodiesel consumption annually increases, the price of oilseed has been raised. With appropriate government's support, farmer will gain benefit from biodiesel promotion. For economical point of view, biodiesel can be directly used for electricity generation via diesel generator. An uncertainty of energy availability is one of the critical problem of renewable energy such as solar, wind and tidal. For example, a solar photo voltage cannot generate electricity during the absence of sun light. Even the energy storage technology has been extensively developed, the storage efficiency, storage time and cost are not economically feasible yet. A hybrid system, which contributes several electricity generation sources, should be considered. Biodiesel is one of the promising choice as a backup energy source since it can be stored for a longer period. This sustainable electricity generation is suitable for remote area where the national grid does not connect and the demand of electricity is still low. The access of electricity use results to an improving quality of life for people in a rural area.

2.1.2. Disadvantages of Biodiesel

2.1.2.1. Food and feed shortage

In order to fill the engine tank, million acres of forest and agricultural areas must be sacrificed. Producing biodiesel from edible feedstock diverts the food and feed supply, reducing the food and feed security. Moreover, the price of food and feed stock are likely to get an impact from the expansion of biodiesel production. Increase in biofuel crop price had an effect on the supply of the low net return crop such as hay. This might affect to cattle and dairy section (Acheampong et al., 2011). Moreover, this impact does not limit within only one certain area. Saunders et al. (2009) reported that the expansion in biofuel crop production in USA had an impact on agricultural and livestock section of New Zealand. Eventually, without caution on expanding biodiesel production, this may impact on the depletion of global edible oil supply.

2.1.2.2. Land use change

Concerning in environmental quality, biodiesel can reduce CO_2 emission, but enormous land have to be used to grow the biodiesel feedstock. According to US fossil fuel consumption in transportation section, 24% of the US total crop land will be needed for growing palm oil, the highest oil yield among terrestrial oil-crop, to meet 50% of the transport fuel demands (Chisti, 2007). This land requirement increases based on the oil yield of different biodiesel feedstock as presented in Table 2.2.

Table 2.2 Comparison of oil yield and land requirement for supporting 50% of all transportation need in US (Chisti, 2007).

Crop	Oil yield (L/ha)	Land area needed (million ha)	Percent of existing US cropping area
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Oil palm	5950	45	24
Microalgae	58700	4.5	2.5

Moreover, several arguments have been stated that there are the negative effects of indirect land use change due to expanding an agricultural area for growing biofuel crop. Kim et al. (2010) and Havlik et al. (2011) reported that the increase in biofuel crop production affects water irrigation and deforestation leading to decrease in global carbon stock. Furthermore, Silvia et al. (2008), Lankoski and Ollikainen (2009), and Cui et al. (2010) stated that growing biofuel crop had multiple impacts on social welfare and environmental quality, for instance, nutrient and herbicide runoff, GHG emission (from growing biofuel crop), soil erosion by wind and water, carbon sequestration and biodiversity reduction.

2.1.2.3. Limitation on vegetable oil extraction technology

Biodiesel production technology is already matured. Nowadays, we can produce biodiesel from the low quality of oil such as waste cooking oil. The rural community could potentially produce and use their own biodiesel. However, vegetable oil extraction process is considered as the major obstructer in biodiesel production since it has to apply the hazardous n-hexane as the oil extraction solvent. Such a process requires high technologies to ensure the safety and health of the workers, resulting in high investment cost. The n-hexane free process for vegetable oil extraction and biodiesel production may be more attractive, which was the main objective of this study for developing this kind of process (i.e. *in-situ* TE).

2.1.2.4. High production cost

The biodiesel feedstock accounts for up to 80% of biodiesel production cost as presented in Figure 2.2. Government must provide subsidies to help biodiesel compatible with petroleum diesel instead of using these budgets to develop other social welfares.

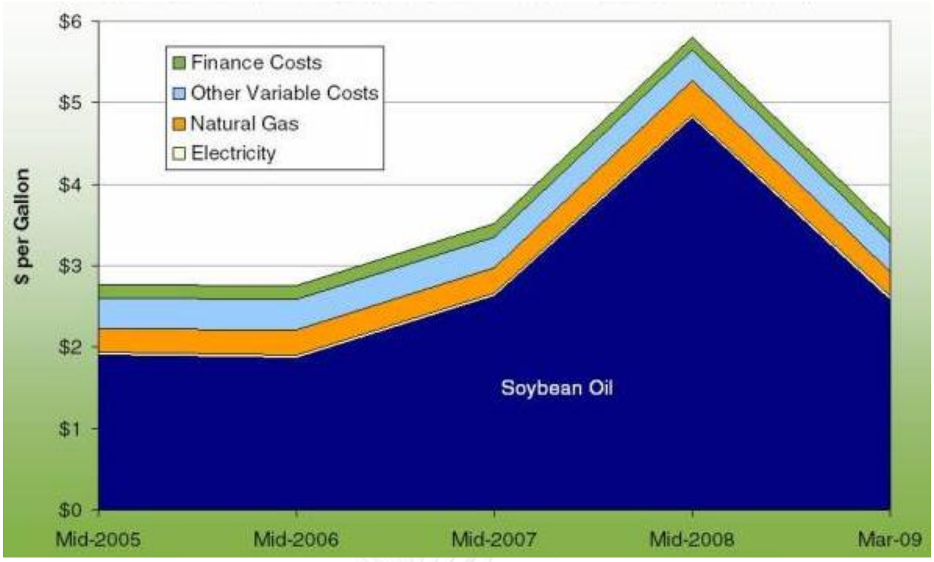


Figure 2.2 Estimated soy biodiesel cost per gallon in USA (Wisner, 2009).

In conclusion, biodiesel feedstock is the main problem of biodiesel production. It is a major cost, increases deforestation, decreases environmental qualities and creates food/feed shortage. Therefore, the ideal biodiesel feedstock should be non-edible and inexpensive in order to make biodiesel production more sustainable.

2.2. Biodiesel Feedstock

Vegetable oils dominate the biodiesel feedstock because they are available and can be produced locally (Yusuf et al., 2011). International Grain Council reveal that the global biodiesel production was mainly produced from edible vegetable oil in 2007. Rape oil was contributed more than 50% of the global biodiesel production, followed by soybean oil and palm oil with 22% and 11%, respectively (International Grain Council, 2008). Based on prediction in Figure 2.3, the proportion of edible oil biodiesel will reduce from almost 90 to 75% by 2019. This is due to the food shortage issue which induces development in biofuel crop, which has high oil content and is considered as non-edible oil, as an alternative biodiesel feedstock. Several biofuel crop show a potential to be a suitable biodiesel feedstock such as jatropha, rubber seed, castor, *Pongamia pinnata,* sea mango (Gui et al., 2008), and especially algae (Huang et al., 2009).

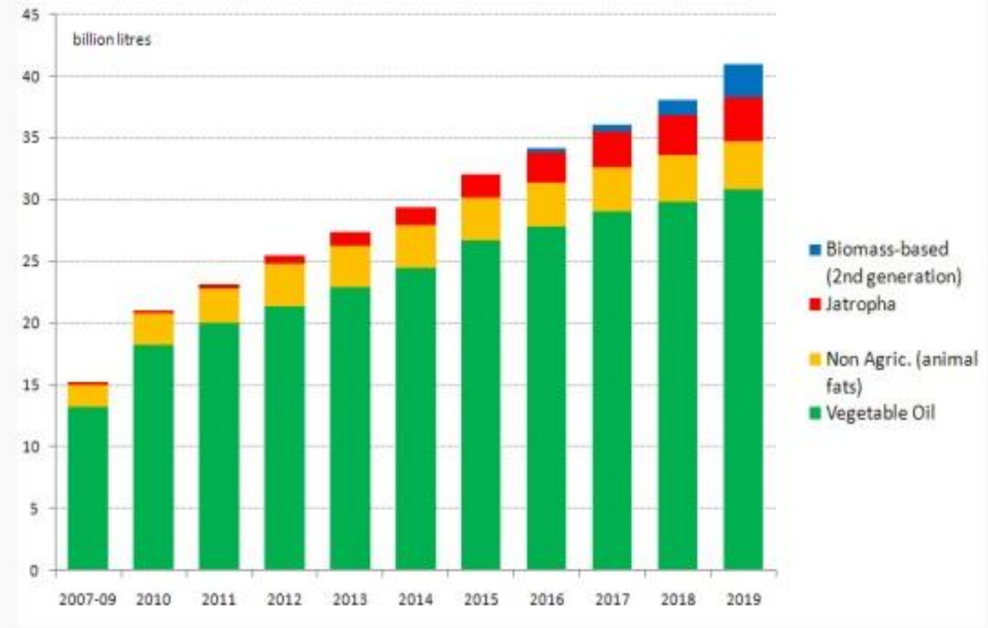


Figure 2.3 Forecast of global biodiesel production between 2010 and 2019 (OECD-FAO Agricultural Outlook, 2010).

In India, a total of 1.72 million hectares of land has been used for jatropha cultivation. Jatropha biodiesel was already commercialized (Gui et al., 2008). Besides of having high oil yield, jatropha plant can grow on poor nutrient soil, even on gravel, sand and saline soil (Carels, 2009). Thus, growing jatropha has less land competitive with edible oil crops which require high quality of soil and water. However, jatropha seed do not ripe at the same time. Thus, hand to hand is only method for harvesting jatropha seed (Carels, 2009). The labor cost is one of a concern aspect, especially in the developed countries where labor cost is high.

Algae is recently an interested non-edible biodiesel feedstock since the enormous higher oil yield compared to any terrestrial oil crops, resulting in lower land requirement for cultivation. A growing of microalgae, however, will require only 1 to 3% of the US total crop land to produce the same amount of biodiesel (Chisti, 2007). Although the high oil yield of microalgae might reduce the impact of food shortage and land use change, the high cost of cultivation and oil extraction are ultimately problems of this marine oil-crop. The palm biodiesel costs roughly 0.66 USD/L; while the microalgae biodiesel costs 2.80 USD/L in 2006 (Chisti, 2007). Moreover, microalgae biodiesel is very sensitive to oxidation during storage since most of fatty acids are polyunsaturated fatty acids with four or more double bonds (Demirbas and Demirbas, 2010).

In spite of biofuel crop, the current interest in non-edible biodiesel feedstock directs to a waste from other sections known as “waste-to-energy” concept. Waste cooking oil (WCO) from household, restaurant and food industry is one of the promising biodiesel feedstock since it has less or even no impacts on food shortage and land use change problems. The WCO is any vegetable oils and/or animal fats that have been used in cooking of food, which is not suitable for human consumption. WCO is a common problem for an urban area since it is directly discharged into drains or sewers. An accumulation of WCO in sewers blocks the water flow leading to flooding in many cities. It also causes several problems in municipal waste water treatment. With the increase in biodiesel production, WCO can be a promising alternative biodiesel feedstock. Even though, a total amount of WCO is insufficient to solely support the currently biodiesel production, using WCO for biodiesel production is a good strategy to simultaneously solve the WCO problem as well as energy shortage. More than 15 million tons/year of WCO is generated from the selected countries as shown in Table 2.3. The price of WCO is very low compared to edible oils. In 2012, the market value of WCO from soybean oil, known as yellow grease, was sold at 955 USD/ton; while crude degummed soybean oil was 1,136 USD/ton (The Trade News Service, 2012). This lower price induces the interest of using WCO as biodiesel feedstock. However, the costly refining step is required to treat WCO prior to biodiesel production due to its high water content and acid value.

Table 2.3 Waste cooking oil generated from selected countries (Gui et al., 2008).

Country	Quantity (million tons/year)
China	4.5
Malaysia	0.5
United States	10
Taiwan	0.07
European	0.7-1.0
Canada	0.12
Japan	0.45-0.57

Consequently, each biodiesel feedstock has its advantages and disadvantages. Thus, diversified resources for biodiesel feedstock is necessary to achieve an annually increase of biodiesel production with low impacts on the environment and economic. In this work, we focused on soybean and spent coffee grounds (SCG) as the biodiesel feedstock used in the *in-situ* TE process. Soybean is considered a commercial feedstock and able to be cultivated in Thailand. It contains high oil content with a good oil quality. In spite of soybean oil product, the soy protein is the high value product. In our point of view, soybean is a more suitable edible biodiesel feedstock compared to the others. In case of SCG, it is considered as the waste from the coffee industry which contain similar oil content to soybean. However, its oil contains high acid value which required a pretreatment process (Vardon et al., 2013). Thus, producing biodiesel using soybean and SCG via the *in-situ* TE process would be worth to investigate.

2.2.1. Soybean

Soybeans contain highly amount of protein (40% w/w) and oil (18-20% w/w). The amino acids and fatty acids in soybean are good for human diet. Moreover, high proportion of polyunsaturated fatty acids (oleic and linoleic) in soybean oil are suitable to produce cold resistant biodiesel as presented in Figure 2.4. The world soybean consumption has been annually increased. In 2010, soybean dominated the world protein meal consumption with 69% and was the second of edible oil with 29% (USDA, 2012). The productions of soybeans and soybean oil are driven by a demand of soy protein in livestock industry (Gunstone et al., 2000).

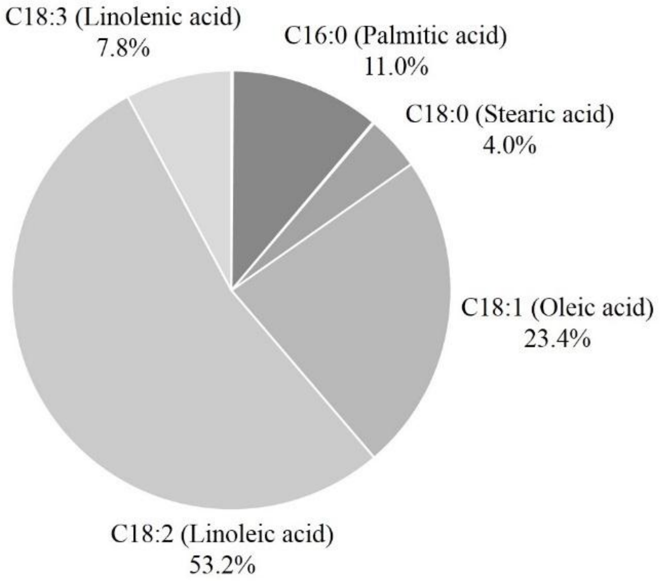


Figure 2.4 Fatty acids composition in soybean oil (% w/w) (Gunstone et al., 2000).

Soybean oil is generally used in several food applications including cooking and salad oils, margarine and shortening, mayonnaise and salad dressing. A soybean lecithin from refining process is commonly used as emulsification agent. Tocopherols and sterols, the main antioxidant in soybean oil, are used in nutrition-supplement and pharmaceutical industries (Gunstone et al., 2000). The presence of tocopherols in biodiesel is able to increase the oxidative stability of biodiesel product (Hass and Scott, 2007).

Since a growing of interest in biodegradable material and high price of petroleum oil, several industrial sections are used vegetable oil as their raw materials such as biodegradable grease, vegetable oil composites material, printing ink, paint and coatings, surfactant, vegetable oil-based engine oil and lubricant (Erhan, 2005). Among them, soybean oil has been intensively used for biodiesel production as alternative to petroleum diesel oil. Biofuel support policy is one major which induces this annually increase of soybean oil production presented in Figure 2.5. The global soybean oil production in 2012 is 150% greater than that of 1999.

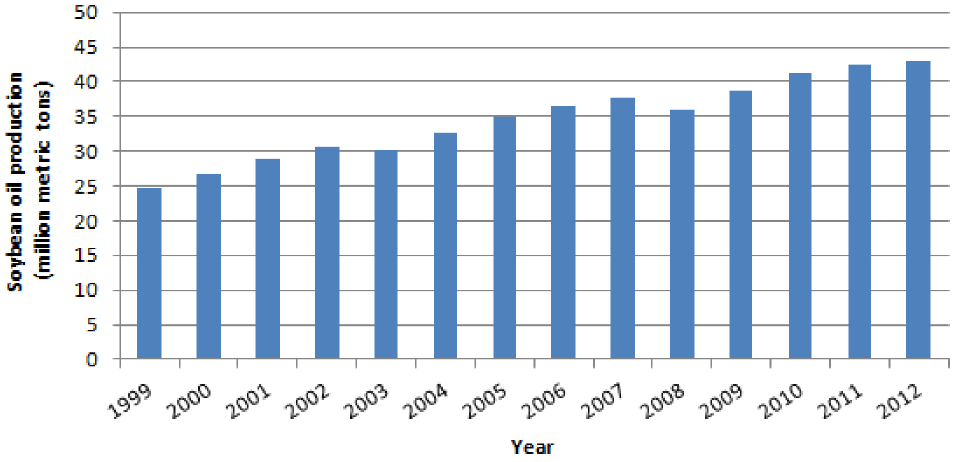


Figure 2.5 Global soybean oil production between 1999 and 2012 (USDA, 2012).

Growing of soybean consumption of Thailand, including soybean meal and oil, has increased. However, Thai domestic soybean production has annually decreased which currently accounted for only 10% of the total supply due to the poor production yield. Soybean oil is responsible for 25% of total vegetable oil consumption in Thailand, which is the second after palm oil with 65%. The production of soybean oil is dominated by the demand of soybean meal as the feed for livestock section because soybean meal accounts for 60% of the soybean crushers' total revenue (Preechajarn, 2012). Therefore, this is a limitation of soybean oil's availability for biodiesel production. In addition, the cost of soybean oil production is fluctuated from unexpected climate changes such as drought and flood in 2011. The soybean crushers are significantly impacted from the increase in production cost since the retail price of soybean oil is controlled by a Thai government policy (Preechajarn, 2012).

In our perspective, using soybean oil for biodiesel production should reduce the impact from the fluctuate price of soybean oil. With government subsidies and taxes reduction, the soybean crusher may get better profit from producing and selling the biodiesel, instead of soybean oil. Thus, the biodiesel process is considered as an alternative choice. Furthermore, Thailand currently has the largest soybean crushing capacity in Southeast Asia with 10,000 metric tons/day followed by Vietnam with 4,000 metric tons/day. In coming of ASEAN Economic Community (AEC) in 2015 should make Thailand become the major supplier of soybean meal and oil for other ASEAN countries (Preechajarn, 2012). This opportunity should guarantee the bright future of soybean oil as biodiesel feedstock in Thailand as well.

2.2.2. Spent coffee grounds (SCG)

SCG, a waste from the coffee industry, has up to 20% oil content (Kondamudi et al., 2008). More than 9 billion tons of coffee were consumed in 2014 (International Coffee Organization, 2016); and global coffee production and consumption have been increasing annually. Approximately 50% of the total coffee consumption was from instant coffee (Ramalakshmi et al., 2009). With the availability of more than 4 billion tons annually of SCG waste from the instant coffee industry, SCG is therefore an attractive non-edible feedstock for the biodiesel industry.

Several studies have shown that biodiesel of adequate quality can be produced using SCG from local coffee shops via conventional biodiesel production processes at a small scale (<100 g SCG per batch) (Vardon et al., 2013; Al-Hamamre et al., 2012). In conventional biodiesel processes, the SCG biodiesel is synthesized via transesterification (TE) using SCG oil extracted by n-hexane solvent (Vardon et al., 2013; Al-Hamamre et al., 2012; Kondamudi et al., 2008). Moreover, the defatted SCG after the oil extraction process could be used as biochar, fertilizer, bioethanol and material composite since it has high heating value and comprises of abundant cellulose, hemicellulose and lignin (Vardon et al., 2013; Kwon et al., 2013; Rocha et al., 2014) as shown in Table 2.4. Also, SCG is an abundant source of bioactive products such as antioxidant, anti-tumor, anti-inflammatory and anti-allergenic (Ramalakshmi et al., 2009).

Table 2.4 Nutrient composition and high heating value of SCG and defatted SCG (Vardon et al., 2013).

Nutrient composition (%)	SCG	Defatted SCG
Crude protein	15.4	18.2
Crude lipid	16.2	0.3
Neutral detergent fiber (hemicellulose, cellulose and lignin)	45.2	58.9
Acid detergent fiber (cellulose and lignin)	29.8	40.2
Ash	1.8	2.4
High heating value (MJ/kg)	23.4	20.1

However, the SCG biodiesel has low cloud point (i.e. high melting point); and it is very sensitive to oxidation. These are due to the high percentage of saturated fatty acid (>40%) and polyunsaturated fatty acid (>40%) in SCG oil as shown in Figure 2.6. Also, the initial water content in SCG is very high with >70% by mass, which potentially reduces the quality of SCG oil due to hydrolysis during transportation and storage. To prevent oil hydrolysis the wet biomass must be stored at frozen temperature and/or dried to a moisture content < 5% by mass (Go et al., 2016).

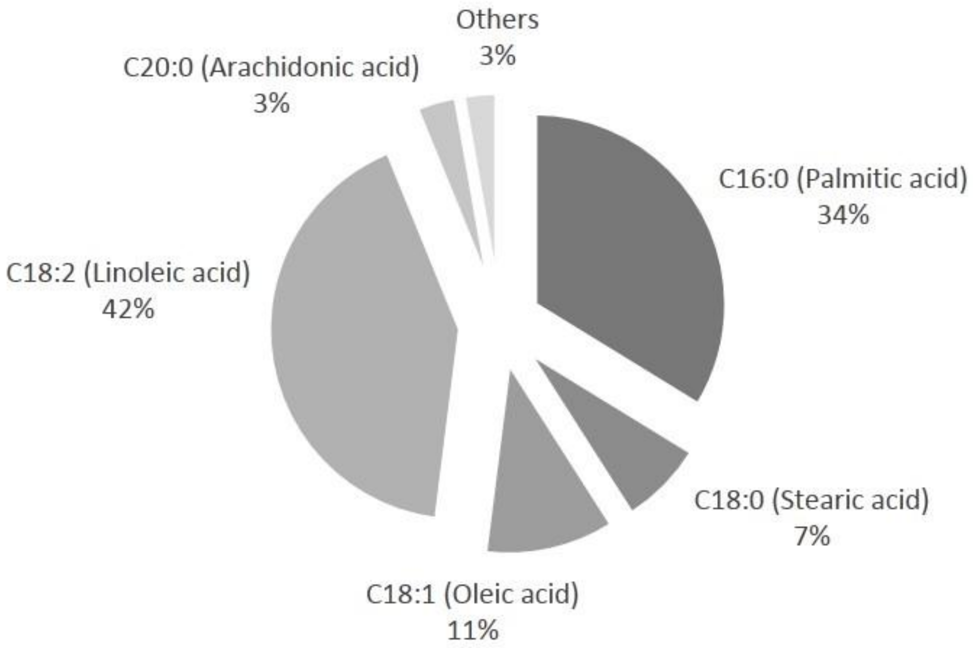


Figure 2.6 Fatty acids composition in SCG oil (% w/w) (Vardon et al., 2013).

One of the challenges of using SCG or its oil in TE is its high acid value between 7 – 13 mg KOH g/oil (Al-Hamamre et al., 2012; Vardon et al., 2013), which results from the roasting and coffee brewing process, as well as from the wet storage. This high acid value neutralizes the alkaline catalyst used during the biodiesel production; hence lowering biodiesel yield. Therefore, pretreatment to reduce the acid value of SCG (deacidification) is a very important step in developing a successful *in-situ* TE process.

2.3. Biodiesel Production

2.3.1. Conventional Biodiesel Production

After harvesting, the oilseeds are transported from agricultural site to central facilities for oil extraction and refining process, and then the refined oil is conversed to biodiesel via TE reaction by catalyzed alcohol as presented in Figure 2.7. The crude biodiesel product, which consists of biodiesel, glycerol and exceed methanol with catalyst, must be purified to meet the ASTM D6751 standard as mentioned in Table 2.1. Gravity separation is commonly applied to separate biodiesel from glycerol and exceed methanol with catalyst. The glycerol and exceed methanol with catalyst bottom phase is distillated for glycerol and methanol recovery. The biodiesel phase is treated by acid water to neutralize the exceed alkali catalyst and remove the residual glycerol. Finally, the moisture content of refined biodiesel is reduced by thermal treatment or applying water adsorption material such as anhydrous sodium sulfate (Van Gerpen and Knothe, 2005).

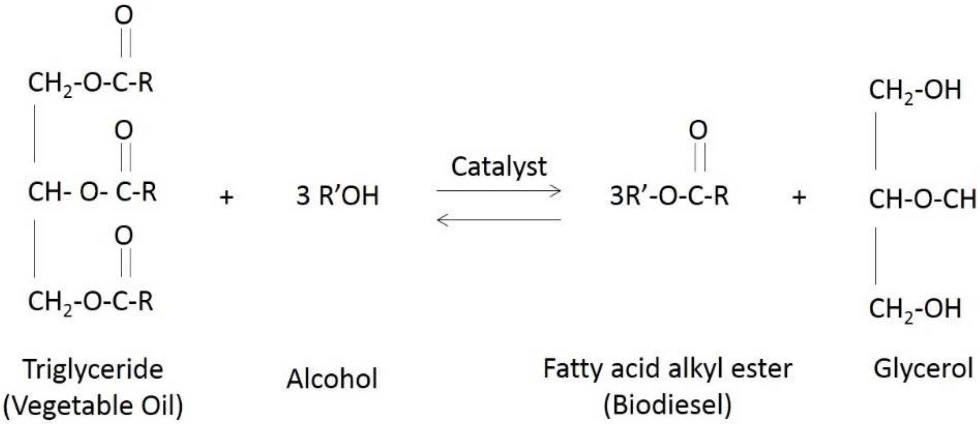


Figure 2.7 TE reaction for biodiesel production.

Based on the catalyst, the biodiesel production is classified into two types which are alkali and acid catalyst. An alkaline catalyst is commonly used in biodiesel production (Figure 2.7) since it provides the higher reaction rate with lower alcohol and catalyst requirement than those of the acid catalyst reaction (Knothe et al., 2005). However, a limitation of alkaline catalyst is the contribution of soap formation once the biodiesel feedstock contains high moisture content and free fatty acids (FFA) as shown in Figure 2.8. The presence of soap formation during the process causes several problems, for example: (1) it makes a difficulty to clearly separate the biodiesel from glycerol phase, (2) promoting the hydrolysis reaction of biodiesel during the storage, resulting in increasing acid value of biodiesel, and (3) potentially clog the filter and nozzle of diesel engine. The suggestion level of FFA content in vegetable oil should be <1 %w/w and the moisture content should be kept as low as possible (Knothe et al., 2005).

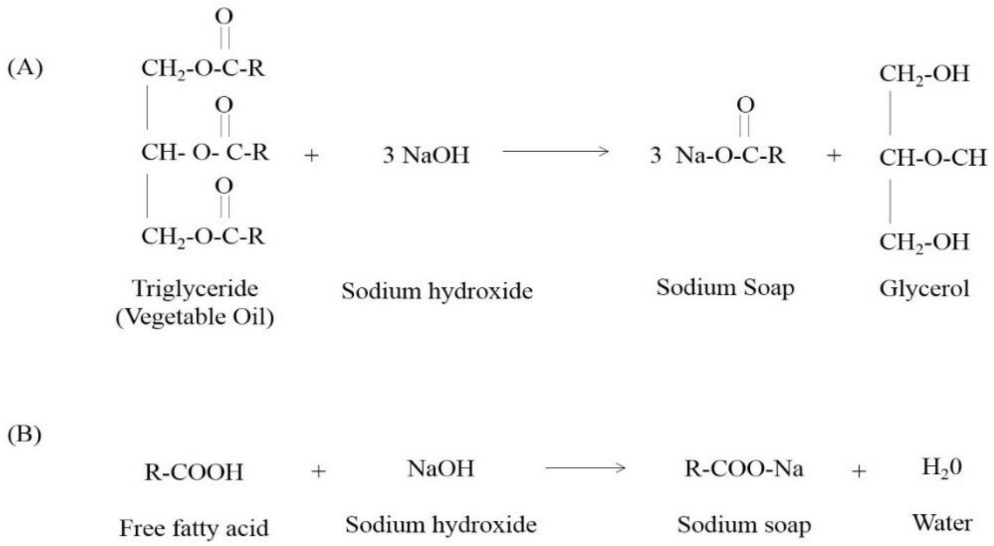


Figure 2.8 Contribution of soap formation during alkaline TE reaction due to the presence of water (A) and FFA (B).

In a case of acid catalyst, it has lower reaction rate and required larger amount of alcohol than those of alkaline catalyst since it is comprised of two steps which are (1) the triglyceride is hydrolyzed to FFA (Fig. 2.9A), and (2) the FFA is conversed to biodiesel via esterification reaction (Fig. 2.9B). Nevertheless, the acid catalyst biodiesel reaction is tolerated to water and FFA. Therefore, it is commonly used as the pretreatment step to reduce high FFA content in vegetable oil, such as palm oil, jatropha oil, SCG oil and wasted cooking oil, prior to alkaline TE reaction (Van Gerpen and Knothe, 2005; Wan Omar et al., 2009; Worapun et al., 2010; Berchmans and Hirata, 2008).

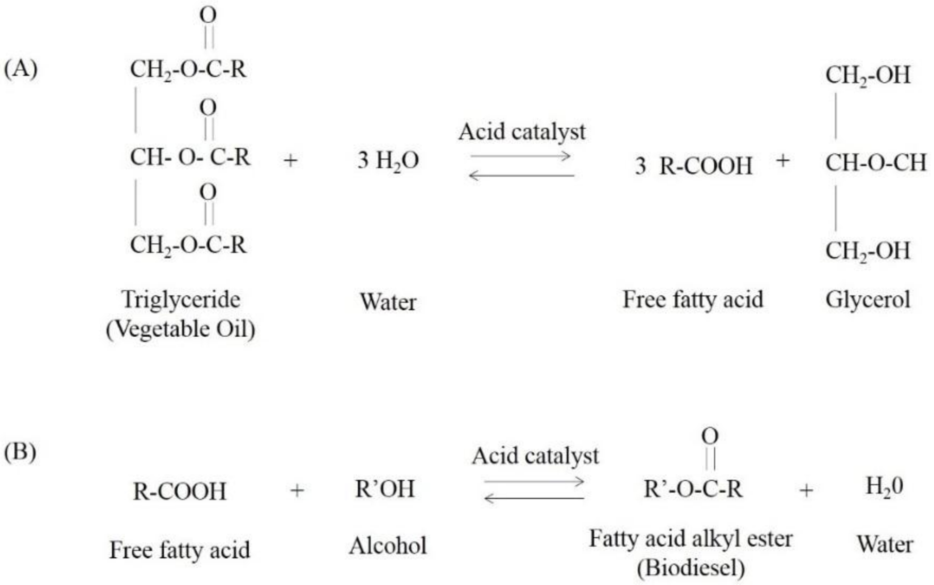


Figure 2.9 Two steps of biodiesel production via acid catalyst consisted of hydrolysis of triglyceride to FFA (A) and esterification of FFA to biodiesel (B).

Among alcohol, methanol is commonly used to produce biodiesel (i.e. fatty acid methyl ester) due to low cost and high TE reaction rate. However, the poor solubility of methanol in vegetable oil is a critical problem. The non-homogeneous mixing leads to incomplete TE reaction. High temperature and intensive physical mixing are required to ensure the biodiesel product quality. Co-solvent is used to increase solubility of methanol in vegetable oil. Tetrahydrofuran is extensively used for the TE acceleration by reducing time from 1 h to less than 5 min with more than 98% biodiesel yield (Çağlar, 2007; Van Gerpen and Knothe, 2005; Mohammed-Dabo et al., 2012; Mahajan et al., 2008).

2.3.2. *In-situ* TE Process

Instead of using vegetable oil, the oilseeds are directly used as biodiesel feedstock in *in-situ* TE. It is a simultaneously process of biodiesel synthesis and solvent extraction using catalyzed alcohol as biodiesel reactant and extraction solvent (Figure 2.10). In short, it is a reactive extraction process which have biodiesel and glycerol as the products. The benefits of this method are to eliminate the use of hazardous n-hexane in vegetable oil extraction and reduce the size and complexity of production system as shown in Figure 2.11. Furthermore, the *in-situ* TE process is able to establish near by the agricultural site (i.e. on-site in-situ TE) which makes local people produce and use their own biodiesel for agricultural machinery, irrigation pump and electric generator, especially the remote areas where national grid does not connect yet. In case of industry, the *in-situ* TE process provided an opportunity for the industries to set up an on-site biodiesel production using their waste as the biodiesel feedstock, resulting in improve the waste management plan and add value to the waste. More detail regarding to co-benefit of biodiesel was described in *"Advantages of Biodiesel."*

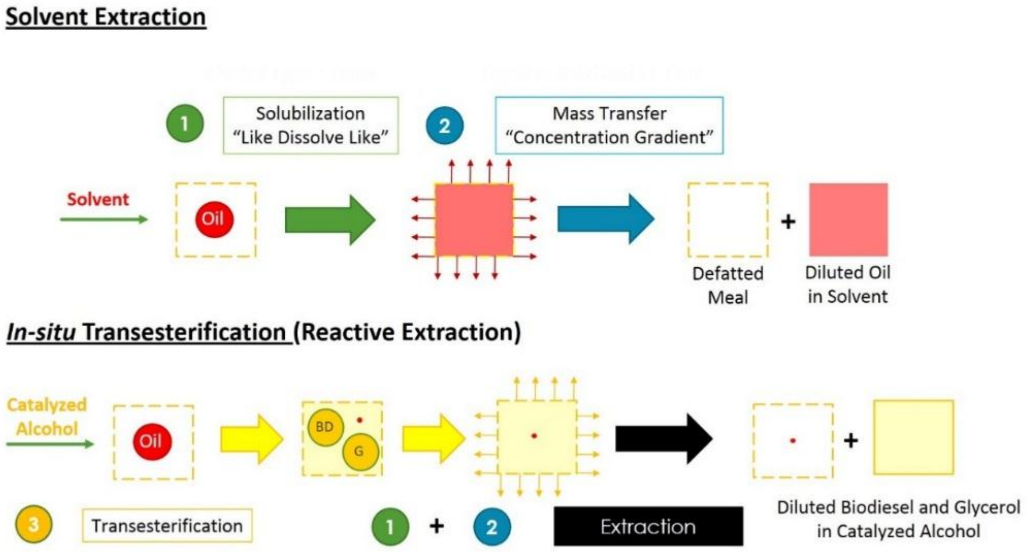


Figure 2.10 Diagram of solvent oil extraction (A) and the in-situ TE process (B). BD is biodiesel and G is glycerol.

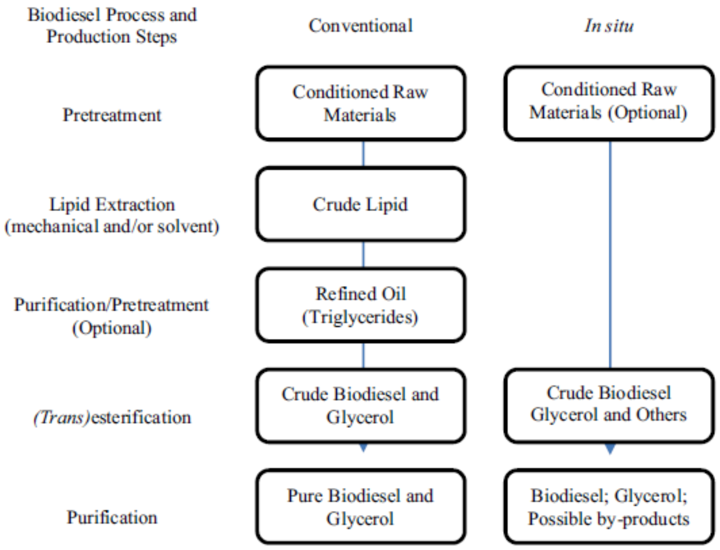


Figure 2.11 Comparison of processing steps between conventional and *in-situ* TE (Go et al., 2016).

Several researchers have proposed the optimal conditions of the *in-situ* TE process as presented in Table 2.5. However, methanol which commonly used in conventional biodiesel production was found not be applicable for the *in-situ* TE due to the poor oil solubility of methanol. The larger amount of methanol loading and much longer operation time were required to ensure the biodiesel yield and quality, compared to those of conventional TE reaction. With larger methanol loading used in the *in-situ* TE process, the reversible TE reaction would push forward to produce biodiesel and glycerol rather than reverse to triglyceride (Figure 2.7). Hence, the problem of *in-situ* TE might be the poor accessibility and interaction of catalyzed methanol to entrapped triglyceride inside the oilseed. Hence, establishing an optimized alcoholic extraction process may be a determining factor for the application of *in-situ* TE. Several factors relating to extraction performance and TE reaction were considered in this study. More detail regarding to each factor were exhibited in subsequence section.

Table 2.5 *In-situ* TE conditions of various oilseeds.

Reference	Hass and Scott (2007)	Georgogianni et al. (2008)	Qian et al. (2008)	Haagenson et al. (2010)
Oilseed	Soybean	Cottonseed	Cottonseed	Canola
Water Content (% w/w)	2.6	Not Report	< 2.0	1.0
Range Size (mm)	Not Report	Not Report	0.3-0.335	0.297-0.841
Alcohol	Methanol	Methanol	Methanol	Methanol
Alcohol to Oilseed Ratio (mL/g)	2.4:1	2.5:1	6.34:1	7.5:1
Catalyst (% w/v)	0.32 (NaOH)	1.6 (NaOH)	0.32 (NaOH)	0.51 (KOH)
Temperature (°C)	25	60	40	60
Time (h)	10	0.33	3	6
Mixer	Shaker	Agitator	Agitator	Water Bath Shaker
Biodiesel Yield (%)	97	93	98	>80

2.3.3. Crucial Factors of *In-situ* TE Process

2.3.3.1. Polarity of alcohol

A like-dissolved-like concept is commonly applied to select the suitable solvent for extracting the interest substance, which was triglyceride in this study. The relative polarity is one of an index to compare the polarity with water, whose relative polarity is equal to 1. Triglyceride has a low relative polarity, thus it is less soluble in methanol with 0.762, leading to poor oil extraction yield. The lower relative polarity of ethanol (0.645) and isopropanol (0.546) (Marcus, 1992) are worth to be investigated in the *in-situ* TE process. As our best knowledge, there is still lack of information regarding to using ethanol in the *in-situ* TE process, and applying neat isopropanol has not been evaluated yet.

2.3.3.2. Acidity of alcohol

In a case of alkaline catalyst which was used in this work, alcohol plays a role as hydrogen donor to alkaline catalyst such as sodium hydroxide. Without catalyst, only alcohol cannot directly convert triglyceride to biodiesel, except using supercritical methanol fluid (Madras et al., 2004). The product of this reaction is alcohol salt (i.e. metal alkoxide) which reduces activating energy of TE reaction, and then triglyceride can be converted to biodiesel. Therefore, the acidity (pKa) of alcohol is a very important parameter for preparing this alcohol salt. The lower the pKa, the easier the hydrogen is donated for contributing alcohol salt formation. Among alcohol, methanol is the best biodiesel reactant since its pKa is the lowest with 15.5, following by ethanol with 16 and isopropanol with 17 (Dewick, 2006). In a case of isopropanol, it is difficult to react with sodium hydroxide to form sodium isopropoxide due to its high pKa value. Applying high temperature with reflux are suggested to generate sodium isopropoxide from sodium hydroxide with isopropanol (Wang et al., 2005). Another approach is to use sodium metal in place of sodium hydroxide since sodium metal is a stronger base; and it is able to prepare the sodium isopropoxide under the mild conditions (Tuntiwiwattanapun et al., 2016).

2.3.3.3. Alcohol to oilseed ratio

According to our preliminary study, alcohol to oilseed ratio or liquid to solid (L-S) ratio should be exceed 2 mL to 1 g of oilseed to ensure the well-mixing. At this L-S ratio level, the alcohol to triglyceride mole ratio is extremely higher than 6 to 1, which is the recommendation level (Van Gerpen and Knothe, 2005). Therefore, the TE reaction of *in-situ* TE might not be affected by L-S ratio. However, it is the crucial factor of a mass (triglyceride) transfer between oilseed and alcohol phase. The more difference in oil concentration between liquid and solid phase, the higher the amount of oil can be extracted. This phenomena is known as concentration driving force effect (Meziane and Kadi, 2008). Methanol, which has poor oil solubility, might require higher L-S ratio in *in-situ* TE process to extract triglyceride from oilseed compared to ethanol and isopropanol.

2.3.3.4. Catalyst type and concentration

As mentioned previously, the reaction rate for producing biodiesel using alkaline catalyst is faster than that of acid catalyst. Therefore, only alkaline catalysts were applied in this study. Insufficient catalyst concentration leads to incomplete TE reaction. In a case of conventional TE reaction using methanol, 1% w/w of catalyst (sodium hydroxide and potassium hydroxide) based on triglyceride weight is suggested (Van Gerpen and Knothe, 2005). For the *in-situ* TE process, this catalyst concentration level is insufficient to complete the TE reaction. Based on the literatures exhibited in Table 2.5, the catalyst concentration should exceed 0.32% w/v in methanol. However, applying high concentration of alkaline catalyst contribute the soap formation which affects the biodiesel and glyceride separation (Freedman et al., 1984; Hass et al., 2004). Compared between sodium metal and sodium hydroxide, applying sodium metal provides better biodiesel yield since it does not generate water during alcohol salt generation as shown in Figure 2.12.

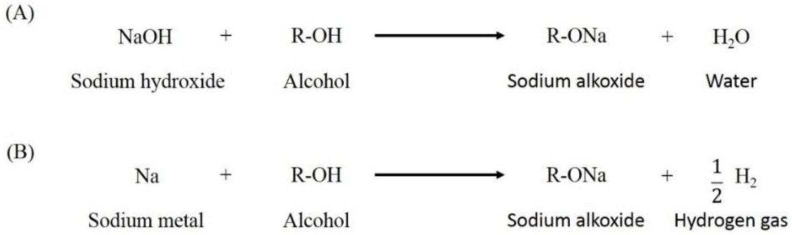


Figure 2.12 Chemical reaction for preparing sodium alkoxide by dissolving sodium hydroxide (A) and sodium metal (B) in alcohol.

2.3.3.5. Reaction time

Two simultaneous processes occur during *in-situ* TE: (1) a rapid washing process to extract and transesterify oil located on or very near the surface of oilseed, and (2) a slower diffusion-limited process to extract and transesterify oil entrapped within the oilseed matrix (Zakaria and Harvey, 2014). In order to reduce reaction time, several factors were considered, including increase oil solubility in solvent, reduce the distance between catalyzed alcohol and triglyceride (i.e. oilseed size reduction), increase temperature, apply high L-S ratio and use high shaking speed.

2.3.3.6. Reaction temperature

A higher reaction temperature can increase solubility of vegetable oil in alcohol to ensure the complete TE reaction and extraction performance. Moreover, at high temperature, the viscosity of alcohol and oil are reduced, so the biodiesel produced from trapped oil inside the oilseed is easier to be extracted. Hence, elevating process temperature can enhance the performance and reduce the reaction time of *in-situ* TE (Hass et al., 2004).

2.3.3.7. Oilseed's properties

As described earlier, oilseed is directly used as biodiesel feedstocks in the *in-situ* TE process, therefore the quality of oilseed significantly affects to performance of *in-situ* TE process. The oilseeds are generally ground into small particles which increases surface area, reduce distance between oil and solvent and disrupt the oil bearing cell inside the oilseeds (Booth, 2004). The recommend particle size for oilseed extraction is between 0.21-0.42 mm (Do and Sabatini, 2010; Kadioglu et al., 2011; Naksuk et al., 2009).

The FFA content in oilseeds is indicated the quality of oil for biodiesel production since FFA react with alkali catalyst, contributing soap formation as shown in Figure 2.8. The FFA content should not be exceed 1% w/w of the extracted oil (Van Gerpen and Knothe, 2005). The high FFA oilseeds, such as jatropha and palm, are suggested to apply acid catalyst rather than alkali catalyst.

Prior to *in-situ* TE process, moisture removal is recommended to ensure the biodiesel yield and quality. Presence of water contributes the soap formation under the alkali condition used in *in-situ* TE (Figure 2.8). In order to reduce the moisture content, heat is commonly applied to evaporate the moisture. For *in-situ* TE process, the moisture content of oilseed should keep below 2% w/w (Hass and Scott, 2007).

2.4. Design of Experiment and Statistical Analysis

Design of experiments (DOEs) were originally developed for modeling fitting of physical and numerical experiments. The objective is to select the points (independent factor; x) where the response (dependent factor; y) should be evaluated for serving the purpose such as screening important factor and optimizing condition (Bezerra et al., 2008). First of all, the DOE (mathematical model) is selected to describe the situation under study. Then, an experimental design is generated to collect the data in an efficient way to fit these data to DOE. Finally, the data are collected and analyzed by selected DOE. The full factorial design is one of DOE which is commonly used to evaluate the relevant of factors, interaction among factors and optimize the conditions. However, it requires a large number of experiment. For example, 81 of experiments are required when 4 factors with 3 levels (3^N; N=4) are accounted in full factorial design. Thus, in this dissertation, we applied other DOEs which can trim down the experiment, while still preserved sufficient data for screening important factors and optimizing conditions. The Statistica Program (version 12, Statsoft, Tulsa, OK) was applied to generate DOE and analyze the data in this dissertation.

2.4.1. Taguchi Orthogonal Array Design (Taguchi)

Taguchi method uses a special set of array called orthogonal array which contains the level combinations of the independent factors for each experiment (Table 2.6). Therefore, this special set can reduce number of experiment, but still provide the full information of all factors that affect the response. For example, only 9 experiments are required when 4 factors with 3 levels are accounted in Taguchi method (array L9 in Table 2.6). In this DOE, it assumes that the main effect of the independent factors on response are separated, thus there is no any interactions among the independent factors. Taguchi method analysts the data using signal to noise ratio (S/N ratio). Ideally, the noise from uncontrollable factor must be keep as low as possible to minimize the error, so the results will respond to only independent factor. There are several S/N ratio conditions based on applications such as “Smaller-the-Better” for minimizing the defects, “Larger-the-Better” for maximizing the yield and “Nominal-the-Best” for selected the certain value. After data analyzing based on S/N ratio conditions, the result will show the effectiveness of each independent factor on response (Singh et al., 2012). Finally, it should be noted that the main propose of using Taguchi method in this study is to screen the crucial factors on *in-situ* TE process for further optimization.

Table 2.6 The experimental set used in Taguchi orthogonal array design.

Array	Number of runs	Max number of factor	Number of factors with these levels			
			2	3	4	5
L4	4	3	3	-	-	-
L8	8	7	7	-	-	-
L9	9	4	-	4	-	-
L12	12	11	11	-	-	-
L16	16	15	15	-	-	-
L'16	16	5	-	-	5	-
L18	18	8	1	7	-	-
L25	25	6	-	-	-	6
L27	27	13	-	13	-	-
L32	32	31	31	-	-	-
L'32	32	10	1	-	9	-

2.4.2. Central Composited Rotatable Design (CCRD)

CCRD is one of a well-known second order model (quadratic model) of response surface methodology (RSM), which is comprised of first order model (2^N; min (-1) and max (+1) value) augmented with central (mean (0) value) and axial ($\pm\alpha$ value) points. The central point in 2^N model can be used for evaluating curvature (non-linear relationship), and the axial points are essential for determining a critical point (maximum, minimum and saddle) (Bezerra et al., 2008). Value of α can be calculated using equation 2.1.

$$\propto = Number\ of\ Factor^{1/4}$$ Equation 2.1

Due to its second order property, CCRD can significantly improve the optimization process, while the first order model (linear model) encounter the lack of fit problem because of interaction among factors and surface curvature. The number of experiment of CCRD is dramatically lower than that of full factorial design (3^N). For example, 16 of experiments are required when 3 factors with 5 levels are accounted in CCRD as shown in Figure 2.13. It should be noted that applying a large number of independent factor in CCRD might be difficult to identify and control the small contributions from each factor (Bezerra et al., 2008). The screening of independent factor is required to determine which of those present more significant effect. In this dissertation, Taguchi method was applied, and then two or three most important factors were accounted in CCRD for process optimization.

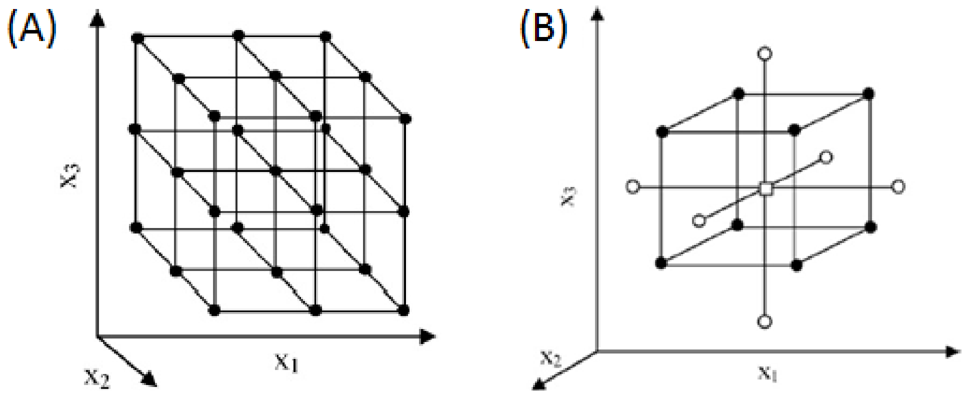


Figure 2.13 Experimental design based on three factors of (A) full factorial design with 3 levels (-1, 0, +1) and (B) CCRD with 5 levels ($-\alpha$, -1, 0, +1, $+\alpha$). The black dot was factorial point, the square was central point, and the white dot was axial point.

2.5. Life Cycle Assessment

Life cycle assessment (LCA) is a wildly used environmental assessment tool to evaluate the impacts of product/service has on the environment over its entire life-cycle, from the raw material extraction; through the manufacturing, packaging and the marketing processes; the use, reuse of the product; and until its eventual recycling or disposal as the waste (Sonnemann et al., 2004). LCA has been used for several purposes, for example:

- Compare alternative choice.
- Identify points for environmental improvement (hotspot).
- Contribute to understanding of the environmental impacts from human activities.

- Generate the whole picture of interactions between product/service and environment.
- Provide support information for decision-makers to select the most efficient solutions for improving the sustainability.

However, it is not necessary to conduct LCA for the entire life-cycle of product/service (i.e. cradle-to-grave). In many cases, this kind of assessment is applied to a single process such as comparing the energy usage and environmental impacts between different biodiesel productions approach using the same biodiesel feedstock in this study (i.e. gate-to-gate). Therefore, setting goal and scope of LCA is the most important of the four main steps as shown in Figure 2.14. The more detail of each step was presented in the subsequence sections.

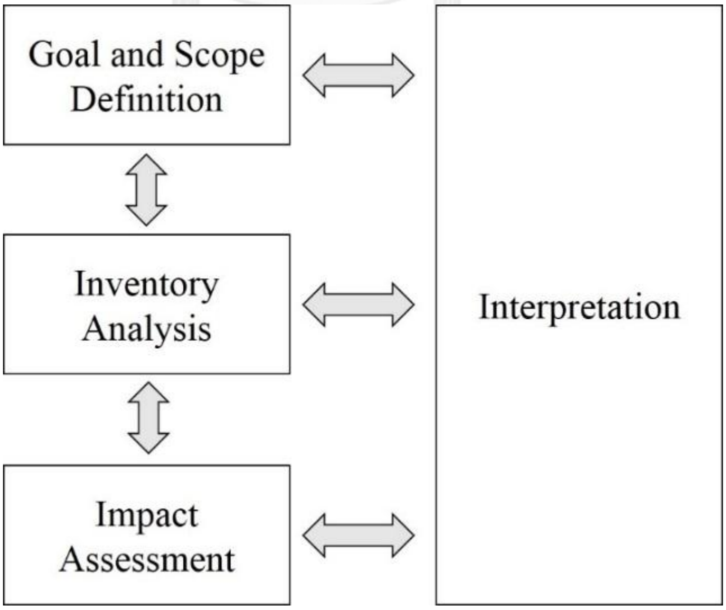


Figure 2.14 Step of conducting LCA.

2.5.1. Goal and Scope Definition

Goal and scope definition is the important step in LCA since it provide the direction and scope of the LCA study. In order to carry out the goal and scope several tasks must be completed, including:

- Define the objective of this LCA study.
- Select the functional unit.
- Set up the boundary.

2.5.2. Inventory Analysis

Inventory analysis is a step in which all environmental loadings and their effects generated by the product/service during its life-cycle are collected. After define goal and scope of LCA study, the data of the unit processing within a product system is collected, and related them to the functional unit. The process diagram is used for systematic data collection (Figure 2.15). However, in a very common cases more than one product/service are produced during the process. Thus, allocation in environmental loading should be conducted in the LCA study. There are several allocation approaches including: mass-based, volume-based, energy-based and economic-based allocations.

In this study we applied mass-based allocation since it is relatively easy to apply and generates a reasonable result (Pradhan et al., 2011). The functional unit was 1 kg biodiesel product. So, we had to calculate the biodiesel fraction of each step exhibited in Figure 2.15. For example, in drying and transportation step the fraction of biodiesel was 0.166 (i.e. 1 x 0.92 x 0.18 x 1). Therefore, the energy usage for producing biodiesel during drying and transportation step was calculated by multiplying its biodiesel fraction (0.166) with the total energy usage in drying and transportation step. The more detail of calculation was presented in chapter 3.

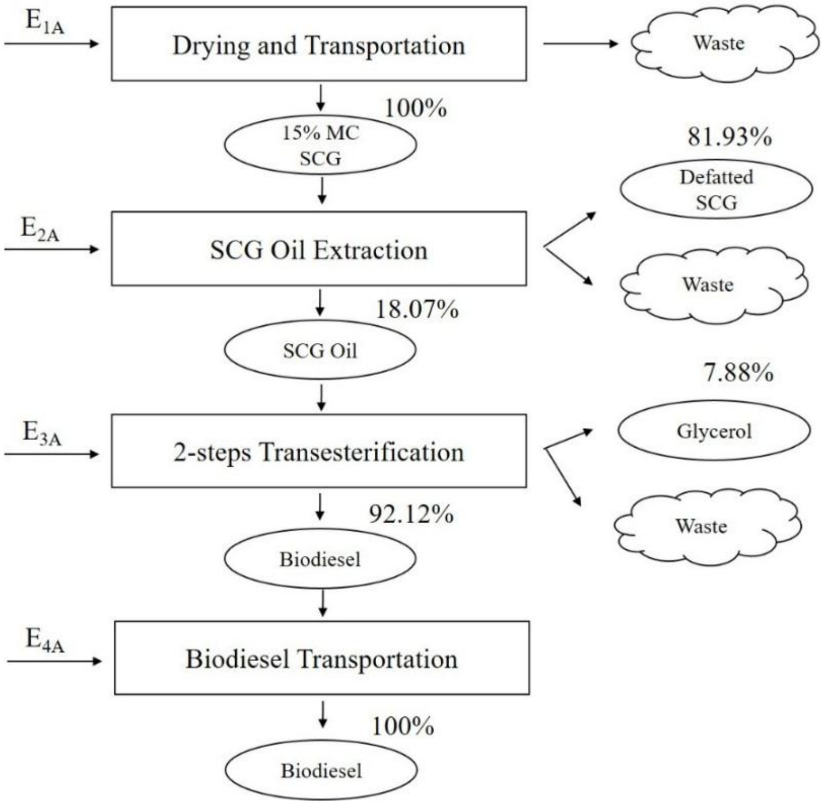


Figure 2.15 Example of process diagram for SCG biodiesel production according to mass-based allocation approach.

2.5.3. Impact Assessment

The inventory analysis provides the basically of a quantified list of environmental loads such as raw material consumption, air and water emission and waste generation. However, the environmental impact associated with them is not evaluated yet. The impact assessment is introduced as the third step of LCA, which makes the results from inventory analysis more understandable in terms of human health, availability of resource and environmental quality. Finally, the results from impact assessment are used in the interpretation step.

Impact assessment is comprised of two mandatory steps which are (1) classification to classify the pollutant to the impact categories and (2) characterization to calculate the effect of those pollutants on each impact category (midpoint and damage indicator). In this study, the IMPACT 2002+ model was used to assessment the impact of the SCG biodiesel production process. The midpoint and damage indicator (endpoint) of this approach was presented in Figure 2.16. In addition, there are optional elements in terms of (1) normalization to group up the relative impact category and (2) weighting to comparison the impact across their category.

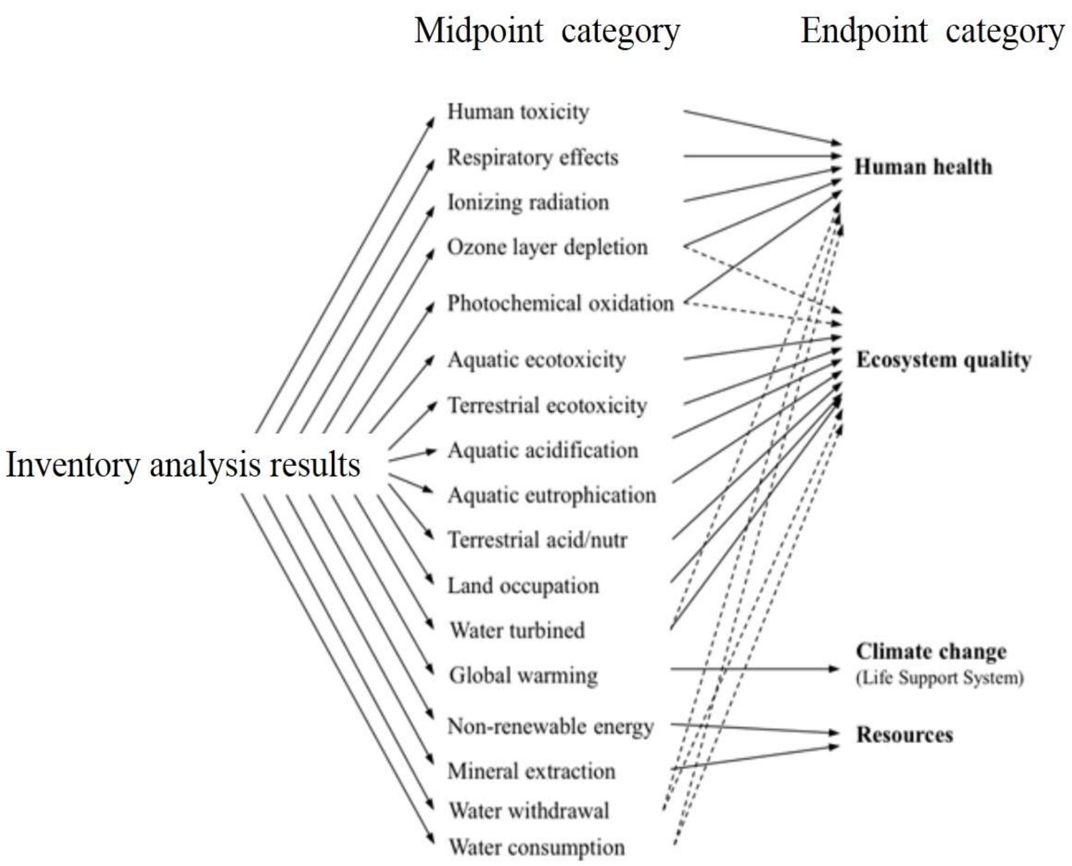


Figure 2.16 Framework of impact assessment according to IMPACT 2002+ method. http://www.quantis-intl.com/en/impact-2002

2.5.4. Interpretation

This final step aims to evaluate the results from inventory analysis or impact assessment and compare them with the goal and scope in LCA study. Thus, we can draw up the recommendation and procedure to improve the environmental quality, energy efficiency and cost effectiveness of the product/service. This results may lead to a new iteration round of the study, including adjustment of the goal and scope definition, inventory analysis and impact assessment as shown in Figure 2.14.

CHAPTER 3

METHODOLOGY

3.1. Materials

3.1.1. Chemicals

Methanol (99.9% purity), ethanol (99.8% purity), acetone (99.8% purity), sulfuric acid (H_2SO_4; 96% purity) and n-hexane (98% purity) were purchased from CARLO ERBA Reagents (Italmar Co., Ltd, Thailand). Isopropanol (99.8% purity) and sodium hydroxide (NaOH; analytical grade) were obtained from RCI Lab scan Limited (Thailand). Sodium metal (Na; analytical grade) was purchased from Panreac Quimica Sau (Barcelona, Spain). Phenolphthalein (1%) in methanol were purchased from VWR (Chicago, IL, USA). 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium hydroxide (analytical grade), aqueous ethanol (95%), butylated hydroxyanisole (BHA) and soybean oil standard (analytical grade) were procured from Sigma-Aldrich, Chemical Co. (St. Louis, MO, USA).

3.1.2. Biodiesel feedstock

3.1.2.1. Soybean

The source of soybean (*Glycine max*) in this study was Phetchabun province, Thailand. The initial moisture content of soybeans was 5.1% mass (wet basis). The oil content of soybeans was 23.1% mass (dry basis) determined by Soxhlet n-hexane extraction (American Society for Testing and Materials, 2009). The acid value of the extracted oil was 0.33 mg KOH/g.

Soybeans were ground by a food blender (Kassel model KBL-550, China). Then the ground soybean was fractionated based on the particle size, and only particles between 0.251 and 0.425 mm were retained. The fractionated ground soybean was incubated in a hot air oven at 75 °C overnight to reduce moisture content to 1.7% mass (wet basis), yet retain the quality of the soybean oil (Hass and Scott, 2007).

3.1.2.2. SCG

The SGC was kindly provided by Jacobs Douwe Egberts TH Ltd., which processes coffee beans (*Coffea robusta*) grown in Chum Pon province, Thailand. The initial moisture content of soybeans was 75.0% mass (wet basis). The oil content of SCG was 18.1% mass (dry basis) determined by Soxhlet n-hexane extraction (American Society for Testing and Materials, 2009). The acid value of the extracted oil was 5.93 mg KOH/g.

SCG was sun dried for three days to 30%. The sun dried SCG was further dried in a hot air oven at 105 ˚C overnight. The dried SCG was fractionated into five different size ranges using US sieve sizes No. 12 (1.68 mm), 16 (1.19 mm), 18 (1.00 mm), 40 (0.42 mm) and 60 (0.25 mm). Due to negligible amount of the fraction of particle size <0.25 mm, it was combined with the fraction 0.25-0.42 mm. The SCG fraction that could not pass through the US sieve size No. 12 was ground using a Kassel food blender (model KBL-550, China) and sieved again through the five different sieve sizes. The five fractions from the grinding step were combined with the corresponding fractions from the initial sieve step, and were reported on a percentage mass basis and used in the subsequent process.

3.2. *In-situ* TE

3.2.1. Soybean

The alkaline catalyst was dissolved in alcohol (i.e. methanol, ethanol, isopropanol and methanol-acetone mixture) to prepare the sodium alkoxide solution, and adjusted to concentration to desired level (i.e. "*alcohol + catalyst*" in Figure 3.1). Five grams of dried ground soybean were suspended in prepared sodium alkoxide solution. The suspension was mixed using a shaking water bath shaker (GFL model 1086, Germany) at various levels of temperature, time and shaking speed. The slurry was then filtered through a Whatman® No. 40 paper filter (England) using a vacuum pump (KNF Neuberger model N026.1.2.AN.18, Germany) at 6.0-6.2 kPa. The glassware and solid fraction on the paper filter were rinsed by 10 cm^3 of alcohol.

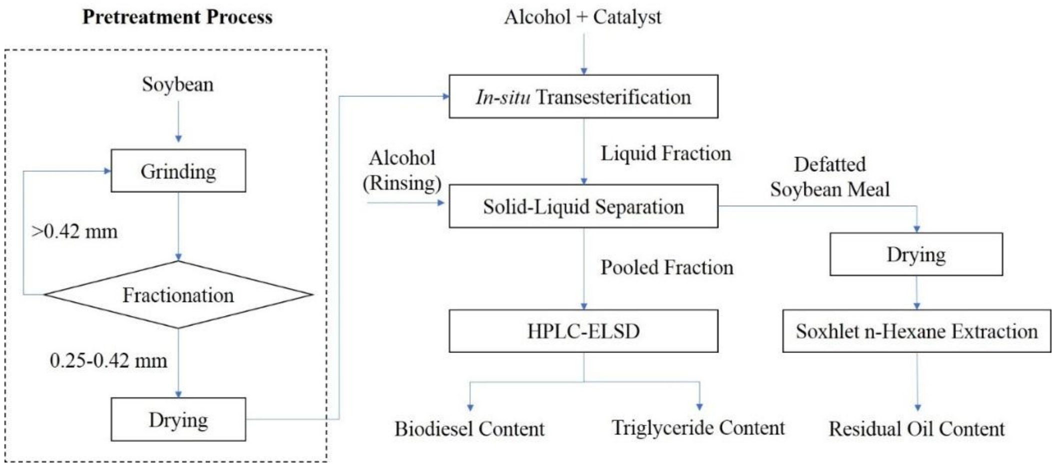


Figure 3.1 Schematic diagram of overall soybean biodiesel production via *in-situ* TE.

The liquid fraction from the *in-situ* TE process and rinse fraction were pooled together ("*pooled fraction*" in Figure 3.1), adjusted to 25 cm^3 by isopropanol, and evaluated for triglyceride and biodiesel contents by HPLC-ELSD. The defatted soybean meal was incubated in a hot air oven at 105 ˚C overnight. Residual oil was then determined using Soxhlet n-hexane extraction described in AOCS official method AM2-93 (American Society for Testing and Materials, 2009).

3.2.2. SCG

Sodium methoxide (alkaline catalyst) solution was prepared by dissolving 2.25 g NaOH in methanol and adjusting the volume to 250 mL. Thirty grams of the selected size of DSCG were suspended in 105 mL of sodium methoxide solution using a three-neck round bottom flask as a reactor (Figure 3.2). The suspension was mixed for 3 h with a mixing speed of 15.7 rad/s using a two-blade propeller at the selected temperature between 30 and 60 °C. After 3 h of operation time, catalyst in the liquid fractions was neutralized by adding 2.76 g of H_2SO_4 (Hass et al., 2004), which was calculated based on the stoichiometry of the NaOH and H_2SO_4 reaction. The slurry was then filtered through a Whatman #4 filter paper under vacuum at 6.0 – 6.2 kPa. The defatted SCG in the glassware and filter paper were rinsed with 15 mL methanol. The filtrate from the *in-situ* TE process and rinse were pooled together and called the "liquid fraction" as exhibited in Figure 3.2. This liquid fraction contains fatty acid methyl ester (biodiesel), excess methanol, glycerol product, water, and sodium sulfate (salt from catalyst neutralization).

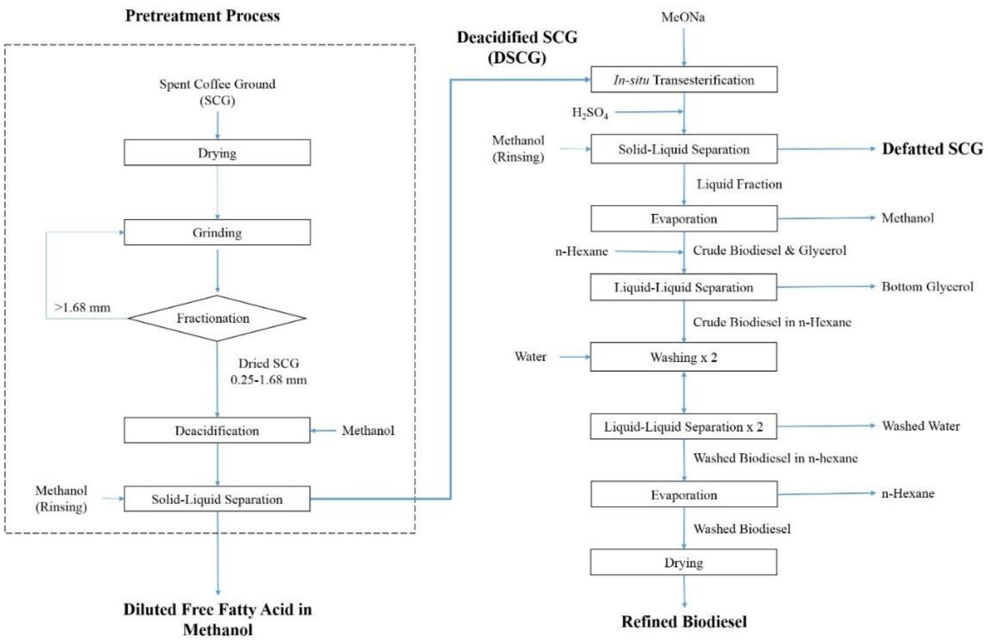


Figure 3.2 Schematic diagram of overall SCG biodiesel production via *in-situ* TE.

The methanol in the liquid fraction was removed by evaporation at 50 ˚C in a water bath with a stream of dry air (-70 ˚C dew point). To ensure complete separation of the 2-phase mixture (crude biodiesel & glycerol), 40 mL n-hexane was added to the liquid fraction after methanol removal, then the mixture was transferred to a 500 mL separatory funnel. The lower glycerol phase was removed and the mixture of biodiesel and n-hexane (crude biodiesel in n-hexane) was washed two times with 25 mL DI water. Before each bottom aqueous wash phase was discarded, the mixture was allowed to settle for 30 min. The washed biodiesel in n-hexane was transferred to pre-weighed amber vials and incubated for 2 h in a water bath at 50 ˚C assisted by dry air purging (-70 ˚C dew point) to facilitate n-hexane evaporation. The refined biodiesel was further incubated in a hot air oven at 105 ˚C for another 1 h to ensure that the biodiesel product met the ASTM moisture specification.

3.3. Analytical Methods

3.3.1 Determination of biodiesel yield

3.3.1.1. Soybean

The biodiesel standards, which were fatty acid methyl ester, fatty acid ethyl ester and fatty acid isopropyl ester, were synthesized following the procedure of Van Gerpen and Knothe (2005), Anastopoulous et al. (2009) and Wang et al. (2005), respectively. The detail of procedure and HPLC-ELSD chromatograph of each biodiesel standard were located in Appendix B. The recovered biodiesel and unreacted triglyceride were calculated from biodiesel and triglyceride contents by HPLC-ELSD multiplied by volume of filtrate and rinse fraction. Finally, the biodiesel yield was calculated using equation 3.1.

$$Biodiesel\ Yield\ (\%) = \frac{BD_{Recovered\ (g)}}{BD_{Theoretical(g)}} \times 100 \qquad \textbf{Equation 3.1}$$

where $BD_{Theoretical}$ was calculated by multiplying conversion factor (1.00, 1.05 and 1.10 for fatty acid methyl ester, fatty acid ethyl ester and fatty acid isopropyl ester, respectively) with weight of dried ground soybean and total triglyceride in dried soybean, quantified by Soxhlet n-hexane extraction and HPLC-ELSD. $BD_{Recovered}$ was calculated by multiplying the biodiesel content of pooled fraction (extracted and rinsed fractions) from the process with its volume.

3.3.1.2. SCG

After biodiesel purification, the refined SCG biodiesel was weighed and the weight was used in equation 3.2 to calculate the biodiesel yield.

$$Biodiesel\ Yield\ (\%) = \frac{Weight\ of\ refined\ biodiesel}{Theoretical\ weight\ of\ biodiesel} \times 100 \qquad \textbf{Equation 3.2}$$

where the theoretical weight of the biodiesel was obtained by multiplying the weight of oil in the DSCG with a conversion factor of 1.00.

3.3.2. Oil and biodiesel quality analysis

3.3.2.1. Oil quality

The Soxhlet n-hexane extraction was used to quantify the total oil and residual oil content of soybean and defatted soybean meal according to AOCS official method AM2-93 (American Society for Testing and Materials, 2009), respectively. Ten grams of dried sample were put into the cellulose thimble, and the thimble was then loaded into the 100 mL of glass syphon. Approximately 150 mL of n-hexane was poured into 250 mL round bottle flask. After assembling these glass wares with condenser tower (Soxhelt apparatus), it was put on the heating mental. The extractions were performed for 6 h with reflux. The n-hexane in extracted fraction was evaporated using a rotary evaporator (Heidolph model Heizbad HB digit, Germany) at 60 °C for 20 min under the vacuum pressure of 6.0-6.2 kPa. The extracted oil was weighted and used to calculate the total oil and residual oil contents.

In cases of SCG, DSCG and defatted SCG, the total oil and residual oil were extracted by n-hexane using an accelerated solvent extraction unit (ASE 200, Dionex Corp, Sunnyvale, CA, USA). Five grams of dried samples were milled in a coffee grinder with 3.5 g diatomaceous earth and then loaded into 11 mL stainless steel cells. The milling and extraction conditions were similar to the conditions in Haagenson et al. (2010). The extractions were performed at 100 °C and 6.7 MPa with a 5 min equilibration time and 3 sets of 10 min static cycles having a 100% flush volume and 60 s purge time with industrial grade N_2. The extracted fractions were automatically collected in pre-weighed vials, and n-hexane solvent was evaporated using a stream of dry air (-70 ˚C dew point). The extracted samples were reground for a second extraction; finally, the total oil content from the two extractions was recorded.

The extracted soybean, SCG and DSCG oil were evaluated their acid value. The fatty acid profile of the soybean and SCG oil were analyzed by GC-FID at The Halal Science Center (Chulalongkorn University, Thailand).

3.3.2.2. Biodiesel quality

The soybean biodiesel was evaluated the quality in terms of kinematic viscosity (40 ˚C), acid value, cloud point and total glycerol content based on ASTM D6751-09 for B100 (American Society for Testing and Materials, 2009).

The quality of the SCG biodiesel was analyzed in terms of kinematic viscosity (40 ˚C), acid value, cloud point, pour point, moisture content, and OSI according to ASTM D6751-09 for B100 (American Society for Testing and Materials, 2009). The total glyceride was evaluated using the SafTest for total glyceride based on the manufacturer's recommendation (MP Biomedical, Solon, OH, USA).

The antioxidant activity of the SCG biodiesel samples was determined using the DPPH antioxidant assay (Naidu et al., 2008). Different concentrations (0.05, 0.1, 0.2 and

5 mg in 1 mL methanol) of biodiesel samples and BHA as reference were added to separate 3 mL vials. A volume of 1 mL of 0.1 mM DPPH in methanol was then added to each vial. The mixtures were vigorously mixed and allowed to stand at room temperature for 30 min. A control sample (methanol in the absence of a biodiesel sample) was prepared as above and used as the baseline correction. The absorbance of each sample at 517 nm was measured using a UV-Vis spectrophotometer. The antioxidant activity was calculated using equation 3.3.

$$Antioxidant\ Activity\ (\%) = (A_0 - A_1)/A_0 \times 100 \qquad \text{Equation 3.3}$$

where A_0 is the absorbance of the control sample and A_1 is the absorbance of the mixture that contained biodiesel or BHA. Then, the EC_{50} value (i.e. the concentration of sample which provides the antioxidant activity at 50%) was determined by non-linear regression analysis.

3.4. Experimental Design

3.4.1. Screening the crucial factors of *in-situ* TE

In this part, Taguchi method was applied to assess the effectiveness on triglyceride extraction and biodiesel yield of 8 variable parameters namely; catalyst type, reaction temperature, time, shaking speed, catalyst concentration, moisture in oilseed, solid-liquid ratio (S/L) and acetone proportion. According to set of experiment in Taguchi method (Table 2.6), the array L18 was the most suitable based on number of parameters in this part. The experimental design was presented in Table 3.1. The problem type of Taguchi method "the larger-the-better" was selected for calculating Eta value (signal to noise ratio: S/N) of triglyceride extraction and biodiesel yield using Statistica program version10. The three most important factors were selected to optimize the conditions in the further experiments.

Table 3.1 Experimental design for screening important factor on triglyceride extraction and biodiesel yield of *in-situ* TE process using Taguchi orthogonal array L18.

Treatment	Catalyst Type	Temperature (°C)	Time (min)	Shaking Speed (rpm)	Catalyst Conc. (% w/v)	Moisture in soybean (% w/w)	Solid-Liquid ratio (g/mL)	Acetone (% v/v)
1	Na (metal)	30	60	125	0.125	1.15	1/4	0
2	Na (metal)	30	120	150	0.25	1.86	1/3	50
3	Na (metal)	30	180	175	0.375	3.53	1/2	75
4	Na (metal)	45	60	125	0.25	1.86	1/2	75
5	Na (metal)	45	120	150	0.375	3.53	1/4	0
6	Na (metal)	45	180	175	0.125	1.15	1/3	50
7	Na (metal)	60	60	150	0.125	3.53	1/3	75
8	Na (metal)	60	120	175	0.25	1.15	1/2	0
9	Na (metal)	60	180	125	0.375	1.86	1/4	50
10	NaOH	30	60	175	0.375	1.86	1/3	0
11	NaOH	30	120	125	0.125	3.53	1/2	50
12	NaOH	30	180	150	0.25	1.15	1/4	75
13	NaOH	45	60	150	0.375	1.15	1/2	50
14	NaOH	45	120	175	0.125	1.86	1/4	75
15	NaOH	45	180	125	0.25	3.53	1/3	0
16	NaOH	60	60	175	0.25	3.53	1/4	50
17	NaOH	60	120	125	0.375	1.15	1/3	75
18	NaOH	60	180	150	0.125	1.86	1/2	0

3.4.2. Optimizing *in-situ* TE for soybean biodiesel production

This part was separated into two main sections which were binary solvent and single alcohol systems used in the *in-situ* TE process. Acetone was used as the co-solvent to improve the oil solubility of methanol (i.e. binary solvent system). Three factors, which were time, liquid to solid ratio (L/S) and acetone proportion, were optimized their conditions according to CCRD. The experimental design of binary solvent system was shown in Table 3.2. In case of single alcohol system, methanol, ethanol and isopropanol were used in the *in-situ* TE process. Two factors, which were L/S ratio and sodium concentration, were optimized their conditions according to CCRD. The experimental design of single alcohol system was shown in Table 3.3.

Table 3.2 Experimental design for optimizing biodiesel yield of the *in-situ* TE process conditions using methanol-acetone mixture as the solvent according to CCRD.

Treatment	Time (min)	Liquid-Solid Ratio (mL/g)	Acetone Proportion (%vol)
1	60.00	3.00	25.00
2	60.00	5.00	75.00
3	120.00	3.00	75.00
4	120.00	5.00	25.00
5	90.00	4.00	50.00
6	60.00	3.00	75.00
7	60.00	5.00	25.00
8	120.00	3.00	25.00
9	120.00	5.00	75.00
10	90.00	4.00	50.00
11	39.80	4.00	50.00
12	140.20	4.00	50.00
13	90.00	2.33	50.00
14	90.00	5.67	50.00
15	90.00	4.00	8.17
16	90.00	4.00	91.83
17	90.00	4.00	50.00

Table 3.3 Experimental design for optimizing biodiesel yield of the *in-situ* TE process conditions using methanol, ethanol and isopropanol as the alcohol according to CCRD.

Treatment	Liquid-Solid Ratio (mL/g)	Sodium Concentration (% wt)
1	3.00	0.30
2	3.00	0.50
3	5.00	0.30
4	5.00	0.50
5	2.59	0.40
6	5.41	0.40
7	4.00	0.26
8	4.00	0.54
9	4.00	0.40
10	4.00	0.40

Despite of these process optimization, there was an extra experiment which studied the effect of aqueous ethanol and temperature on biodiesel yield of *in-situ* TE process by using the optimal conditions of ethanolysis *in-situ* TE process from previous experiment. The experimental design of using aqueous ethanol in the *in-situ* TE process and effect of process temperature on biodiesel yield was exhibited in Table 3.4.

Table 3.4 Experimental design for optimizing triglyceride extraction and biodiesel yield of the aqueous ethanolysis *in-situ* TE at various temperature according to CCRD.

Treatment	Moisture Content in Ethanol (% vol)	Temperature (°C)
1	1.00	40.0
2	1.00	60.0
3	5.00	40.0
4	5.00	60.0
5	0.17	50.0
6	5.83	50.0
7	3.00	35.9
8	3.00	64.1
9	3.00	50.0
10	3.00	50.0

3.4.3. Developing and scaling-up *in-situ* TE process for SCG biodiesel production

The findings and knowledges from *in-situ* TE for producing soybean biodiesel were applied in this part to develop *in-situ* TE for SCG biodiesel production. Even though, soybean and SCG have similar oil content approximately 20% wt, the oil quality of SCG was very poor due to its high acid value up to 7 mg KOH/g. Therefore, deacidification process was required to reduce high acid value in SCG.

3.4.3.1. Developing deacidification

After the sieving and size reduction steps, each SCG fraction was washed with methanol to reduce the high FFA content. In case of optimizing the conditions, five grams of dried ground SCG at various particle sizes were suspended in methanol. The suspension was mixed for 1 h using a 15.7 rad/s mixing speed by water bath shaker (GFL model 1086, Germany) at various levels of L-S ratio and reaction temperature based on CCRD as shown in Table 3.5. The slurry was then filtered through a Whatman® No. 40 paper filter (England) using a vacuum pump (KNF Neuberger model N026.1.2.AN.18, Germany) at 6.0-6.2 kPa. The glassware and solid fraction on the paper filter were rinsed by 10 cm^3 of solvent. Then, the deacidified SCG (DSCG) were dried in a hot air oven at 75 °C overnight. Finally, DSCG were determined its residual oil content by Soxhlet n-hexane extraction (American Society for Testing and Materials, 2009) and acid value. . The selected conditions, which reduced acid value to <1 mg KOH/g with highest residual oil in DSCG, were applied in subsequence experiments, which scaled up to 36 g of SCG loading per batch and used in the *in-situ* TE process.

Table 3.5 Experimental design for optimizing residual oil content in DSCG and acid value of DSCG oil by methanol washing according to CCRD.

Treatment	Liquid-Solid ratio (mL/g)	Temperature (°C)
1	2.00	35.00
2	2.00	55.00
3	4.00	35.00
4	4.00	55.00
5	1.59	45.00
6	4.41	45.00
7	3.00	30.86
8	3.00	59.14
9	3.00	45.00
10	3.00	45.00

3.4.3.2. Effect of particle size and reaction temperature on biodiesel yield

Despite of its high acid value, the particle size of ground SCG in this study was very coarse, which might reduce the biodiesel yield of *in-situ* TE. Thus, the effect of particle size range of ground DSCG and temperature on biodiesel yield of *in-situ* TE were evaluated. The full factorial design with 4 levels of each factor (i.e. 16 experimental batches) was applied in this part. The levels of each factor were exhibited in Table 3.6. The selected conditions were then evaluated the kinematic parameters using biodiesel yield from 11 time points between 0.05 to 3 h of reaction time. Then, the developed process was scaled-up from 30 g of SCG/batch to 4 kg of SCG/batch (pilot scale). Finally, the SCG biodiesel product was evaluated the biodiesel qualities based on ASTM biodiesel standard and the antioxidant activity based on DPPH assay.

Table 3.6 Values of DSCG particle size and process temperature used to optimize biodiesel yield of *in-situ* TE for SCG biodiesel production.

DSCG Particle Size (mm)	Temperature (˚C)
1.19-1.68	30
1.00-1.19	40
0.42-1.00	50
0.25-0.42	60

3.4.3.3. Kinetic model

Biodiesel yields at 11 different time points between 0.01 and 3 h were evaluated using whole DSCG at 50 ˚C. To stop the TE reaction, 2.76 g of H_2SO_4 was added to neutralize alkaline catalyst at the desired operation time; the product was then recovered and refined as described in section 2.3. Then, the biodiesel yields at the 11 time points were used to calculate the kinetic parameters. The kinetic model for the *in situ* TE reaction in equation 3.4 was adapted from the model proposed by Meziane and Kadi (2008) for vegetable oil extraction:

$$Y_t = Y_W[1 - \exp(-k_W t)] + Y_D[1 - \exp(-k_D t)] \qquad \textbf{Equation 3.4}$$

where Y_t is the biodiesel yield (%) at time t (h),Y_W and Y_D are the biodiesel yields at equilibrium from the washing and diffusion processes, and k_W and k_D are the rate constants (1/h) of the washing and diffusion processes, respectively. The underlying assumptions in this model are that the levels of catalyst and methanol are sufficient excess that the rate of *in-situ* TE is primarily controlled by mass transfer phenomena; therefore, the rate is initially controlled by washing (conversion of oil at or near the particle surface), and later by diffusion within particles (Zakaria and Harvey, 2014).

3.4.3.4. Scaling-up SCG biodiesel production

A modified 38 L steam jacketed kettle (GROEN model TD/2-40; Jackson, MS) with an anchor-shaped agitator shown in Figure 3.3 was used as the reactor to prepare biodiesel from 4 kg batches of SCG. The kettle modifications described in Monono et al. (2015) improved the heat transfer, mixing, and containment of the kettle contents during the process. The process steps and operating conditions during deacidification and *in-situ* TE were similar to those used in the small scale process as shown in Figure 3.2. However, the mixing speed was 20.95 rad/s and there was no DSCG drying step to remove residual methanol before the *in-situ* TE process. The DSCG was directly added to sodium methoxide in the kettle once the temperature reached 50 °C. After 3 h of operation time and catalyst neutralization with H_2SO_4, the slurry was separated by filtration through a 57 x 46 cm Whatman #3 filter paper with the aid of a Duo-seal vacuum pump (The Welch Scientific Co., model 1400; Skokie, IL). The reactor and defatted SCG on the filter paper were rinsed with 4 L methanol. The liquid fraction was transferred to an 18 L polyethylene container while waiting for methanol evaporation.

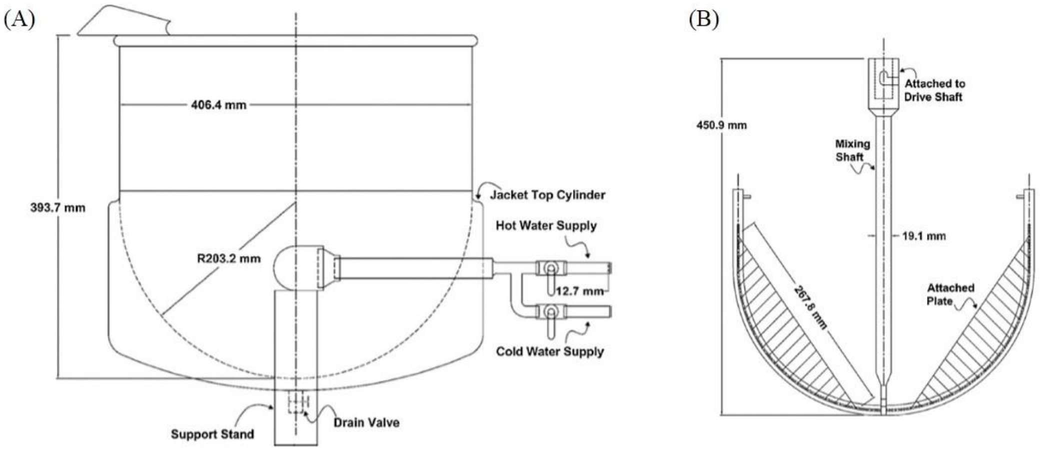


Figure 3.3 A 38 L reactor and its propeller used in pilot-scale SCG biodiesel production via the *in-situ* TE process Monono et al. (2015).

A 12 L Soxhlet unit was used to evaporate the methanol from the liquid fraction. The liquid fraction was divided into three portions of approximately 6 L during methanol removal. The evaporation temperature was set at 90 ˚C, and the condenser temperature was fixed at 4 ˚C. The evaporation time for each fraction was 2.5 h. The total methanol evaporation time was 8 h including the time to remove and add new portion of the liquid fraction. Approximately one kg of 2-phase mixture (crude biodiesel & glycerol) was obtained after this process.

To ensure complete separation, 1 L n-hexane was added to the 2-phase mixture, and the mixture divided into two portions before they were transferred to 3 L separatory funnels. After the glycerol phase was removed, the mixture of biodiesel and n-hexane (crude biodiesel in n-hexane) was washed with 100 mL DI water and allowed to stand for 30 min. Then, the bottom aqueous phase was discarded. This water-wash step was repeated. The washed biodiesel in n-hexane was transferred to pre-weighed amber bottles and incubated under a fume hood for 2 h in a water bath at 50 °C assisted by dry air purging (-70 °C dew point) to facilitate n-hexane evaporation. The refined biodiesel was further incubated in a hot air oven at 105 °C for another 1 h to ensure that the biodiesel product meets the ASTM moisture specification.

3.4.3. Evaluating energy usage and environmental impacts of an on-site SCG biodiesel production via *in-situ* TE process

The developed pilot scale (4 kg SCG/batch) of SCG biodiesel production using *in-situ* TE from previous part was extrapolated to industrial scale level (1,725 kg SCG/batch). Such a process was used to produce SCG biodiesel at an on-site of instant coffee plant (i.e. on-site in-situ TE process). The purposes of this part were to evaluate the energy usage and environmental impacts of on-site in-situ TE process for SCG biodiesel production, and compare the results to those of conventional process based on LCA method. The overview of two different approaches for SCG biodiesel production was exhibited in Figure 3.4.

The system boundary was “gate-to-gate” included SCG pretreatment process at instant coffee plant to SCG biodiesel product. Thus, the cultivation of the coffee bean, coffee roasting and brewing process were not included in this study as well as the use of SCG biodiesel as biofuel. One kg of SCG biodiesel was used as a functional unit. The IMPACT 2002+ in Simapro version 8.0 was used to evaluate the environmental impacts.

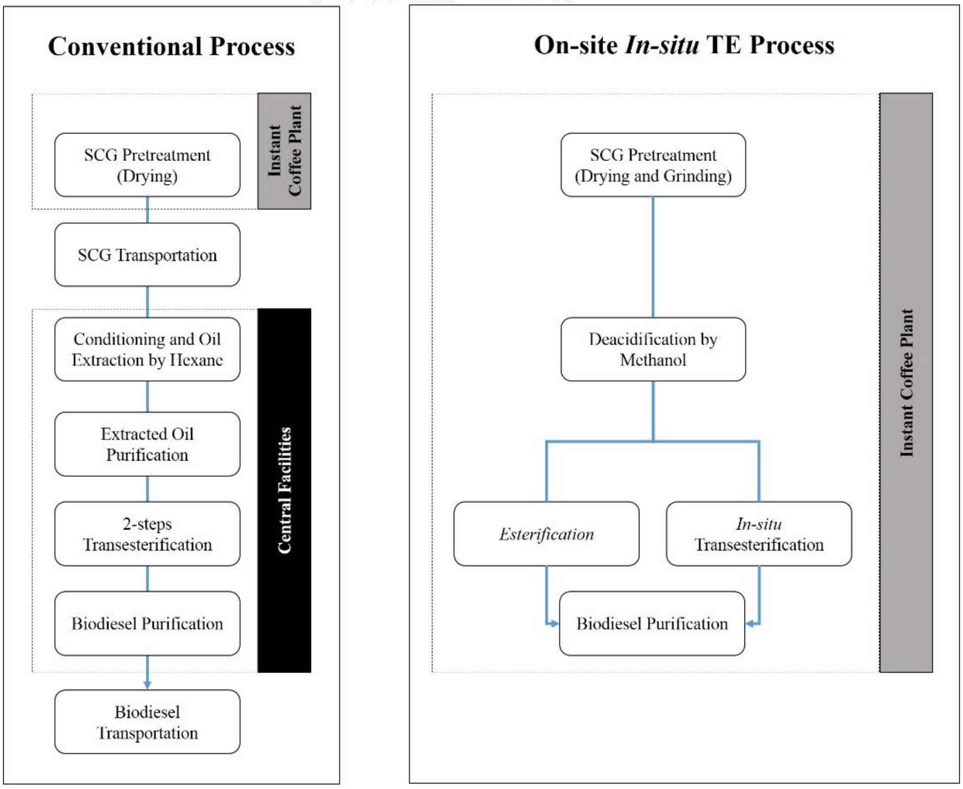


Figure 3.4 A comparison of SCG biodiesel processing steps between conventional and on-site *in-situ* TE process.

CHAPTER 4

SCREENING CRUCIAL FACTORS OF *IN-SITU* TE

The main objective of this part was to evaluate the effectiveness of factors related to triglyceride extraction and biodiesel yield of *in-situ* TE process. Soybean was selected as the biodiesel feedstock in this part due to its high oil quality (i.e. no need for pretreatment process). Since *in-situ* TE is the reactive extraction process, eight factors related to both TE reaction and extraction performance were selected, including: catalyst type, reaction temperature, time, shaking speed, catalyst concentration, moisture in oilseed, S/L ratio and acetone proportion. Taguchi array L18 was selected as DOE with problem type "the larger-the-better", previously presented in Table 3.1. The Statistica program version 10 was used to generate the DOE and analyst the results in terms of Eta value and ANOVA.

4.1. Maximizing Process Conditions by Eta Value

The Eta value (signal to noise; S/N ratio) was calculated based on response factor (i.e. triglyceride extraction and biodiesel yield) of each level of the independent factors. There are several formulas to calculate Eta value based on the goal of the experiment. In this part, the goal of Taguchi method was to maximize the response factors, so "the larger-the-better" problem type was selected, and Eta values were calculated using equation 4.1. For example, to calculate the Eta value of Na (metal), all the response values (*y*) related to Na (metal) (treatment 1-9 in Table 3.1) were used in equation 4.1, so the “n” value was 9 (number of treatment related to Na (metal)). The calculated Eta values of each factor levels on triglyceride extraction and biodiesel yield were shown in Figure 4.1. The Eta value revealed that the biodiesel yield was directly related to amount of triglyceride extraction since they had very similar trends of Eta value as shown in Figure 4.1A and 4.2B. This finding implied that the extraction process might be a determining factor for the application of *in-situ* TE. These Eta values also used in ANOVA test as presented in Table A1 and A2 for triglyceride extraction and biodiesel yield, respectively.

$$Eta\ value\ (S/N\ \ ratio) = -10 \times \log(\sum(1/y^2)/n) \qquad \text{Equation 4.1}$$

where *y* was the response values of level of certain factor, and *n* was the number of experiment related to those level of certain factor.

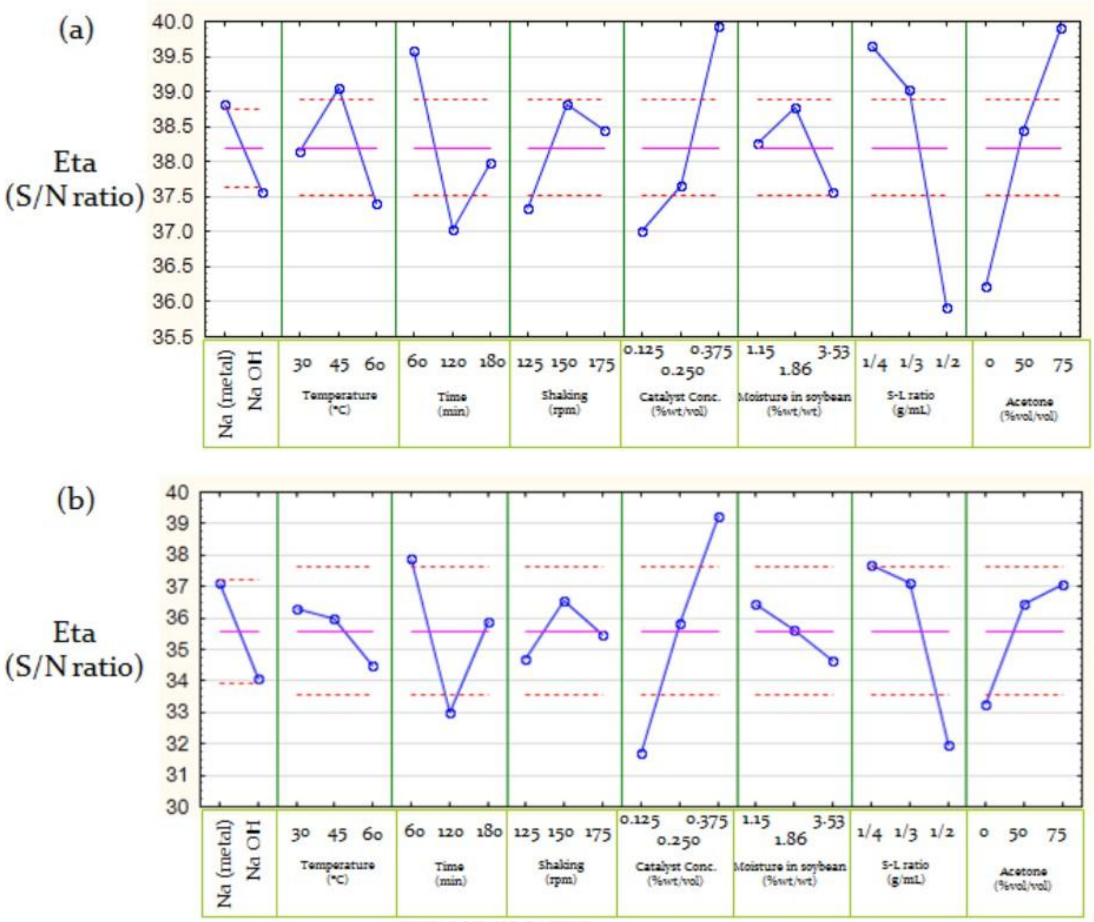


Figure 4.1 Average Eta value by factor levels of triglyceride extraction (a) and biodiesel yield (b). Solid line indicated mean and dash line indicated 2α of standard deviation.

4.1.1. Na (metal) and NaOH

The Eta value showed that Na (metal) was a better catalyst than NaOH in term of both triglyceride extraction and biodiesel yield. This is due to the absence of water as the by-product once using Na (metal) to prepare sodium methoxide (Figure 4.2A and 4.2B). The presence of water during the process enhances the soap formation via saponification reaction (Figure 2.8). The soap product is comprised of free fatty acid with sodium catalyst (i.e. sodium salt of fatty acid), so generating soap during the *in-situ* TE process causes insufficient sodium catalyst and incomplete biodiesel reaction (Tuntiwiwattanapun et al., 2016). The insufficient catalyst due to the soap formation also reduced the triglyceride extraction. The more detail of the phenomena is described below.

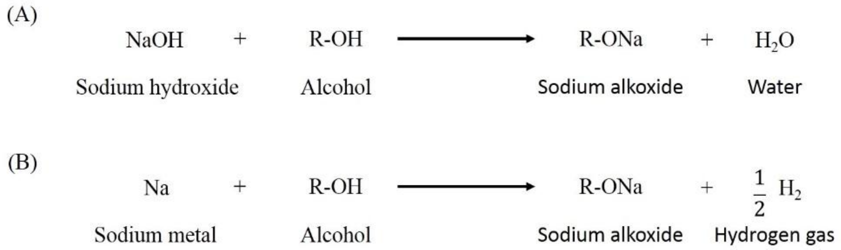


Figure 4.2 Chemical reaction for producing sodium alkaline via sodium hydroxide (A) and sodium metal (B) with alcohol. R was the alkyl group of alcohol.

4.1.2. Temperature

Generally, elevating temperature can enhance both TE reaction and extraction performance. However, with the presence of water from moisture in oilseed and water by-product from NaOH catalyst, it could be a negative effect on triglyceride extraction and biodiesel yield. The maximum Eta values of temperature on triglyceride extraction and biodiesel yield was at 45 °C, and they reduced once the temperature increase to 60 °C. This is because a saponification reaction occurred causing the reduction of triglyceride extraction and biodiesel yield as previously mentioned. Elevating temperature to certain temperature (60 °C in this study) can promote saponification reaction, which competed with TE reaction. The more detail of saponification effect on biodiesel yield was described in the next chapter.

4.1.3. Time

Surprisingly, extending the reaction time of the *in-situ* TE process reduced the triglyceride extraction and biodiesel yield of this study. Commonly, both triglyceride extraction and biodiesel yield should increase and become steady after the equilibrium time (Zakaria and Harvey, 2014). This might be due to the hydrolysis of biodiesel with a presence of alkaline catalyst. The further study on kinetic parameters might reveal this contradict result of our experiment.

4.1.4. Shaking speed

Shaking speed was related to the level of turbulence within the extraction vessel. A sufficient shaking speed has to be applied to ensure a good extraction performance (Tuntiwiwattanapun et al., 2016). This factor is depended on the viscosity of suspended oilseed in solvent and type of mixing (i.e. magnetic stirrer, shaker, impeller and ultrasonication) (Go et al., 2016). In our case, shaking speed at 150 rpm provided the maximum triglyceride extraction and biodiesel yield based on the Eta value.

4.1.5. Catalyst concentration

The Eta value showed that increase catalyst concentration increased both triglyceride extraction and biodiesel yield. The sufficient catalyst concentration was required in the *in-situ* TE process since it is the reactive extraction (Zakaria and Harvey, 2014). Normally, methanol cannot well extract triglyceride due to its low oil solubility. However, once the sodium methoxide (i.e. methanol + catalyst) used as catalyst in the *in-situ* TE process converses the triglyceride into biodiesel and glycerol, which are completely dissolved in methanol. Moreover, the high catalyst concentration can rapid the TE reaction, which reduces the reaction time and temperature of the *in-situ* TE process. However, it might also enhance the saponification (Hass et al., 2004). Consequently, the optimal concentration of catalyst was conducted in the further experiments.

4.1.6. Moisture content in oilseed

As previously mentioned, presence of water affected to both triglyceride extraction and biodiesel yield due to the soap formation generation. The Eta values also showed the negative effect of moisture content in oilseed. Hass and Scott (2007) suggested that the moisture content in oilseed should not be exceed 2% wt to ensure a good performance of *in-situ* TE. Our results agreed well with this suggestion.

4.1.7. S-L ratio

The S-L ratio indicated the alcohol loading in the process. The amount of alcohol used in the *in-situ* TE process (>200 mol methanol to 1 mol triglyceride) was much far higher than the suggested amount of alcohol used in conventional TE reaction (6 mol methanol to 1 mol triglyceride) (Van Gerpen and Knothe, 2005). Therefore, the limitation of S-L ratio was mass transfer between biodiesel and oil in the oilseed to sodium methoxide solution, known as concentration driving force. Eta values indicated that increased in S-L ratio reduced both triglyceride extraction and biodiesel yield since the concentration driving force reduces at higher S-L ratio, especially in S-L ratio at 1/2 g/mL. At this level, the residual oil in defatted soybean meal contained high proportion of biodiesel since the poor extraction performance (data not shown). However, using too low S-L ratio implied that much higher alcohol was required in the *in-situ* TE process, which required tremendous energy to recovery this excess alcohol. Thus, the optimal condition of S-L ratio was determined in the further experiments.

4.1.8. Acetone proportion

Acetone was used as the co-solvent to improve the poor oil solubility of methanol in the *in-situ* TE process. The Eta value exhibited that increase acetone proportion increased both triglyceride extraction and biodiesel yield. Thus, this finding could be used to prove the first hypothesis of this study, described in chapter 1. However, at acetone proportion of 75% vol, the biodiesel yield was not high and uncorrelated to triglyceride extraction (Figure 4.1). This was due to the soap formation. Both Na (metal) and NaOH cannot be dissolved in acetone, so at high proportion of acetone the catalyst precipitation was observed. Applying high proportion of acetone could improve triglyceride extraction, but the precipitated catalyst could be freely reacted with water and generated the soap formation (Figure 4.3). The optimal condition of acetone proportion was determined in the next experiment.

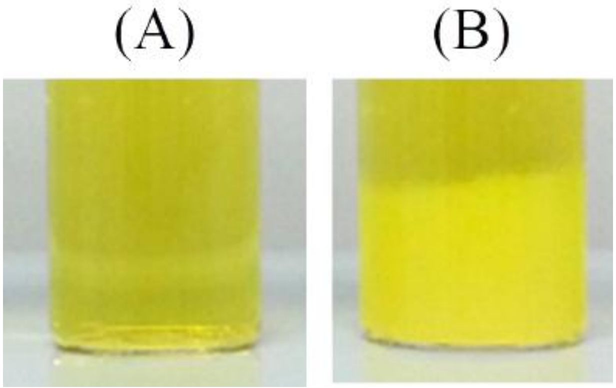


Figure 4.3 Liquid fraction after *in-situ* TE process from treatment 5 (A; 0% vol acetone proportion) and treatment 3 (B; 75% vol acetone proportion). The white turbid bottom phase in B was soap formation.

4.2. Selecting the Most Crucial Factors

All of eight factors significantly affected on triglyceride extraction as shown in Table A1 (p-value <0.05). In case of biodiesel yield, only five factors were considered as significant factor as shown in Table A2 (p-value <0.05) which were catalyst type, time, catalyst concentration, S-L ratio and acetone proportion. The insignificant effect of temperature, shaking speed and moisture content in oilseed might be related to saponification reaction. The further study how to prevent the saponification was exhibited in the subsequent experiment.

The F-value from ANOVA result was used to select the three most crucial factors on triglyceride extraction and biodiesel yield. The higher F-value is the more effect on the response. Based on F-value of ANOVA results, S-L ratio and acetone proportion were the two most important factors on triglyceride extraction of methanolic *in-situ* TE process (Table A1), while the two most important factors on biodiesel yield were catalyst concentration and S-L ratio (Table A2). Hence, S-L ratio, catalyst concentration and acetone proportion were selected to optimize the *in-situ* TE conditions in the subsequent experiments.

4.3. Conclusion

The Eta value from Taguchi method showed that the biodiesel yield was related to the triglyceride extraction. Thus, the extraction conditions may be a determining factor for the application of *in-situ* TE. The soap formation due to saponification reaction during the in-situ TE process should be another concern since it was observed when water and catalyst precipitation presented in the *in-situ* TE process. Elevating temperature improved the biodiesel yield, but with the presence of water and catalyst precipitation, it showed a negative effect due to enhancement of saponification reaction. To prevent saponification was then investigated in the next chapter. According to F-value of ANOVA results, S-L ratio, catalyst concentration and acetone proportion were the three most important factor of *in-situ* TE, and they were used to optimize the process condition in the subsequent experiments.

CHAPTER 5

OPTIMIZING *IN-SITU* TE FOR PRODUCING SOYBEAN BIODIESEL

According to the results from the previous chapter, three factors were selected to optimize the *in-situ* TE conditions including L-S ratio, catalyst concentration and acetone proportion at moderate temperature (i.e. 30 - 45 °C). In this part, two solvent systems were optimized their process conditions which were (1) binary solvent using acetone as the co-solvent to improve oil solubility of methanol, and (2) single solvent using three neat alcohols: methanol, ethanol and isopropanol. The CCRD was applied to design the experiment. Statistica program version 10 was used to optimize the process condition of each solvent system. Moreover, an approach to prevent the saponification was developed in this part using aqueous ethanol as the alcohol. The results from this chapter and chapter 4 were used to develop the *in-situ* TE process for producing SCG biodiesel in the next chapter.

5.1. Optimization of *In-situ* TE Using Methanol with Acetone as Co-solvent

Acetone plays a role as a co-solvent to improve the oil solubility of methanol in the *in-situ* TE process. In order to optimize the process, three factors namely reaction time, L-S ratio and acetone proportion were selected. According to the previous study on screening the crucial factor in chapter 4, the conditions which provided the maximum biodiesel yield were selected for this study: Na (metal) at 0.375% w/v, 45

°C and 150 rpm of shaking speed. Based on CCRD, 17 treatments were required for this optimization. The DOE and biodiesel yield of each treatment were presented in Table 5.1. The results were analyzed for ANOVA test and regression coefficient by software Statistica version 10.

Table 5.1 Design of experiment for optimizing *in-situ* TE using methanol with acetone as co-solvent and their biodiesel yield.

Treatment	Time (min)	Liquid-Solid ratio (mL/g)	Acetone Proportion (%vol)	Biodiesel Yield (%)
1	60.00	3.00	25.00	91.3±2.0
2	60.00	5.00	75.00	73.7±5.6
3	120.00	3.00	75.00	94.8±5.4
4	120.00	5.00	25.00	85.2±2.3
5	90.00	4.00	50.00	87.4±5.0
6	60.00	3.00	75.00	97.0±5.7
7	60.00	5.00	25.00	89.3±3.3
8	120.00	3.00	25.00	101.3±5.1
9	120.00	5.00	75.00	77.3±3.3
10	90.00	4.00	50.00	94.2±0.4
11	39.80	4.00	50.00	87.9±2.8
12	140.20	4.00	50.00	95.1±3.9
13	90.00	2.33	50.00	96.4±1.3
14	90.00	5.67	50.00	82.1±3.3
15	90.00	4.00	8.17	98.0±3.2
16	90.00	4.00	91.83	93.9±3.0
17	90.00	4.00	50.00	97.9±2.6

The ANOVA results reveled that only L-S ratio significantly affected to biodiesel yield (Table A3). These results indicated that the equilibrium time of biodiesel yield might be within 60 min when acetone was used as a co-solvent, and increase in the acetone proportion >25% vol slightly affected to the biodiesel yield. This might be due to the soap formation from the Na (metal) catalyst precipitation at high proportion of acetone as previously mentioned in chapter 4.

The predicted equation by regression analysis was exhibited in equation 5.1 with R^2 0.94. The insignificant factors, which had p-value >0.05, were excluded from the equation. The predicted biodiesel yield was used to generate a contour graph of biodiesel yield as presented in Figure 5.1. The optimal conditions and its biodiesel yield were exhibited in Table 5.2.

$$Biodiesel\,Yield\,(\%) = 32.8 + 0.5X_1 + 20.1X_2 - 2.2X_2^2 + 0.4X_3 - 0.1X_2X_3 \qquad \text{Equation 5.1}$$

where X_1 was reaction time (min), X_2 was L-S ratio (mL/g), and X_3 was acetone proportion (% vol).

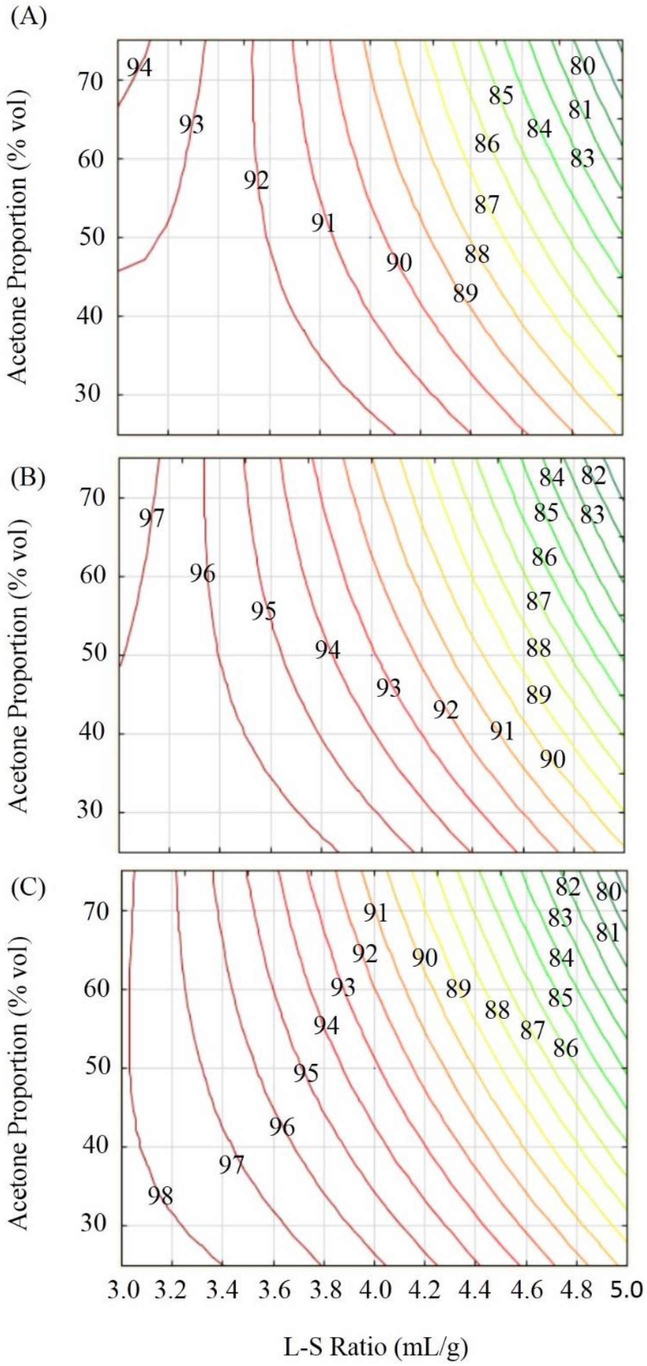


Figure 5.1 Contour plot of biodiesel yield from *in-situ* TE process between L-S ratio and acetone proportion at reaction time of 60 (A), 90 (B) and 120 (C) min using Na (metal) concentration of 0.375% w/v, reaction temperature of 45°C and shaking speed of 150 rpm.

Table 5.2 Optimal conditions of *in-situ* TE process using methanol with acetone as co-solvent and its biodiesel yield.

Parameter	Optimal Conditions
Reaction Time (min)	120
L-S Ratio (mL/g)	3
Acetone Proportion (% vol)	25
R^2 and R^2 Adjust	0.94 and 0.81
Predicted Biodiesel Yield (%)	102
Observed Biodiesel Yield (%)	100 ± 5

According to the results, acetone presented both positive and negative effect on biodiesel yield of methanolic *in-situ* TE process. At low L-S ratio, increase acetone proportion improved the biodiesel yield, but it found decreasing the biodiesel yield at high L-S ratio conditions (Figure 5.1). This might be due to the dilution effect of methanol under the high proportion of co-solvent (Go et al., 2011). Another suspect might be the saponification reaction. Under high proportion of acetone, the catalyst precipitation was observed. According to the reactive extraction concept, firstly the triglyceride must be conversed to biodiesel and glycerol before extraction step, but in the case of using acetone as co-solvent, some triglyceride might be able to be extracted before TE reaction due to the high oil solubility of methanol-acetone mixture. The extracted triglyceride freely reacted with precipitated catalyst and turned to soap formation. This phenomena could be enhanced under the high L-S ratio since the higher concentration driving force increase the triglyceride extraction rate. Therefore, preventing catalyst precipitation was required. The Na (metal) concentration at 0.325% w/v might be too high and precipitated under the presence of acetone co-solvent. The optimum conditions between acetone proportion and catalyst concentration was worth to be investigated in the future.

Nevertheless, 100% of biodiesel yield was achieved under the optimum conditions presented in Table 5.2. Compared to the optimal conditions of the other literature related to methanolic *in-situ* TE process without co-solvent, our process condition as described in Table 2.5 was able to be operated at the rapid reaction time, the lower concentration of catalyst, alcohol loading and reaction temperature. However, the solvent recovery of methanol-acetone mixture might be more difficult and more complicated than that of neat methanol.

5.2. Optimization of *In-situ* TE Process Using Single Alcohol

Methanol, ethanol and isopropanol were used to optimized the *in-situ* TE process by varied the L-S ratio and the Na (metal) concentration. The CCRD was applied to generate the DOE, and Statistica program (version 10) was used to analyze the ANOVA result and regression coefficient. The biodiesel yields of each treatments from three alcohols were presented in Table 5.3. The ANOVA results of biodiesel yield using methanol, ethanol and isopropanol were presented in Table A4, A5 and A6, respectively.

Table 5.3 Biodiesel yield of *in-situ* TE process using three types of alcohol.

Treatment	L-S ratio (mL/g)	Na (metal) Concentration (% wt)	Biodiesel Yield (%)		
			Methanol	Ethanol	Isopropanol
1	3.00	0.30	58.9±0.8	100.9±0.9	101.2±1.4
2	3.00	0.50	59.0±0.2	80.4±1.5	98.9±1.8
3	5.00	0.30	71.7±0.1	85.9±0.9	99.2±0.1
4	5.00	0.50	53.2±3.9	63.8±1.9	65.8±2.8
5	2.59	0.40	50.6±1.8	99.7±0.0	101.5±1.8
6	5.41	0.40	79.9±0.8	72.1±0.5	83.1±0.2
7	4.00	0.26	55.2±0.7	98.7±1.3	105.6±2.6
8	4.00	0.54	43.2±2.0	60.2±1.4	86.6±0.4
9	4.00	0.40	80.7±0.3	75.8±2.2	101.7±0.2
10	4.00	0.40	82.9±1.1	75.9±1.6	99.5±1.7

The ANOVA results indicated that L-S ratio and Na (metal) concentration did not significantly affect to biodiesel yield once methanol was used in the *in-situ* TE process. However, in cases of using ethanol and isopropanol, both factors were significant effects on biodiesel yield. The optimal conditions of each alcohol and its biodiesel yield were summarized in Table 5.4. The predicted equations of methanol (equation 5.2), ethanol (equation 5.3) and isopropanol (equation 5.4) were used to generate the contour plots of biodiesel yield of each alcohol as shown in Figure 5.2.

$$Biodiesel\,Yield\,(\%) = -366.1 + 84.2X_1 - 7.4X_1^2 + 1380.8X_2 - 1537.4X_2^2 - 48.5X_1X_2 \quad \text{Equation 5.2}$$

$$Biodiesel\,Yield\,(\%) = 263.3 - 47.7X_1 + 5.1X_1^2 - 250.7X_2 + 182.1X_2^2 - 4.1X_1X_2 \qquad \textbf{Equation 5.3}$$

$$Biodiesel\,Yield\,(\%) = -88.2 + 62.7X_1 - 4.9X_1^2 + 471.3X_2 - 298.1X_2^2 - 77.8X_1X_2 \qquad \textbf{Equation 5.4}$$

where X_1 was L-S ratio (mL/g), and X_2 was sodium concentration (% w/v).

Table 5.4 Optimal conditions of *in-situ* TE process of each alcohol and its biodiesel yield.

Parameter	Optimal Conditions		
	Methanol	Ethanol	Isopropanol
L-S Ratio (mL/g)	4.48	3.00	3.00
Na (metal) Concentration (% w/v)	0.38	0.30	0.30
Lack of Fit (model evaluation)	Acceptable	Acceptable	Acceptable
Predicted Biodiesel Yield (%)	84	103	100
Observed Biodiesel Yield (%)	87 ± 1	100 ± 1	101 ± 1

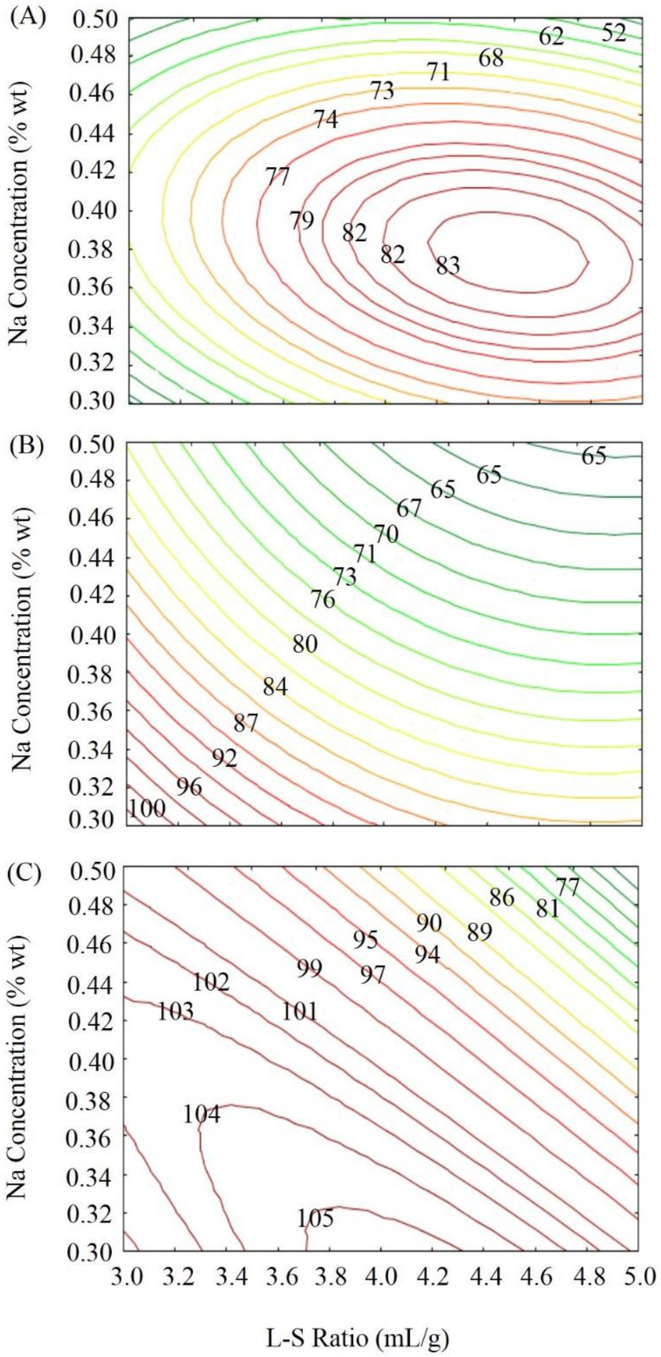


Figure 5.2 Biodiesel yield of *in-situ* TE process between Na (metal) concentration and L-S ratio at 30°C reaction temperature, 60 min reaction time and shaking speed 150 rpm using methanol (A), ethanol (B) and isopropanol (C).

According to the results on biodiesel yields and their optimal conditions, ethanol and isopropanol, which were higher hydrophobic than methanol, provided higher biodiesel yield and used less alcohol loading and lower sodium catalyst concentration than those of methanol. These could reduce the cost of biodiesel production. However, the biodiesel yield declined once the Na (metal) concentration was exceed its optimal condition due to the formation of soap. In order to compare the performance of alcohol used in the *in-situ* TE process, the other parameters should be considered including renewability, toxicity, reaction reversibility, environmental and health concerns, by-product, fuel quality and property and the overall economics of the process (Go et al., 2016).

Both ethanol and isopropanol are renewable alcohol which can be produced by fermentation of biomass. Methanol can be produced by wood gasification; however, the majority of methanol is still produced from petroleum-based (Isayama and Saka, 2008). The toxicity and flammability of ethanol and isopropanol are lower than that of methanol based on NFPA standard. In addition, the produced biodiesel from ethanol and isopropanol have better biodiesel quality, especially fatty acid isopropanol ester which has a superior in cold flow properties (Wang et al., 2005). It should be noted that this was the first report that fatty acid isopropyl ester was produced directly from oilseed (Tuntiwiwattanapun et al., 2016).

Nevertheless, a constraint of using ethanol and isopropanol is a requirement of relatively high energy used in an alcohol recovery step compared to using of methanol. The boiling point, specific heat and latent heat of ethanol and isopropanol are higher than those of methanol. The further study on this issue would be worth to investigate for selecting the most suitable alcohol used in *in-situ* TE.

Additional to the high energy usage in alcohol recovery, ethanol and isopropanol are even more expensive than methanol. The azeotrope effect of these alcohols with water makes the difficulty in removing all of the water from these alcohols. As previously mentioned, the presence of water in the system caused serious problem regarding to soap formation. Therefore, the high technology, such as applying molecular sieve and water absorption, are required to ensure the quality of alcohol and promising biodiesel yield. In this work, the approach to use the aqueous ethanol in the *in-situ* TE process was investigated in the below section.

5.3. Applying Aqueous Ethanol to Produce Biodiesel via *In-situ* TE Process

Due to the negative effect of water in the system which generated soap formation, the approach for reducing this effect was investigated in this study. According to the previous results, elevating temperature enhanced the saponification reaction, therefore operating the *in-situ* TE process at lower temperature might reduce the effect of water in the system. Hereafter, the objectives were to evaluate the effect of moisture content in ethanol and temperature on biodiesel yield and non-reacted triglyceride in biodiesel; and identify the maximum water content in ethanol, which still maintain the biodiesel yield of *in-situ* TE. The optimal conditions of ethanolysis *in-situ* TE from the previous study were applied in this part (Table 5.4). The CCRD was used to generate the DOE and the results were analyzed by Statistica program version 10 in terms of ANOVA test and regression coefficient. Ten treatments were required to optimize the process condition which were presented in Table 5.5 along with their biodiesel yields and triglyceride in biodiesel. The ANOVA results was exhibited in Table A7 and A8.

Table 5.5 Experimental design matrix for optimizing the aqueous ethanolysis *in-situ* TE process and the biodiesel yield of each treatment.

Treatment	Water Content in Ethanol (% vol)	Temperature (°C)	Biodiesel Yield (%)	Triglyceride in Biodiesel (% wt)
1	1.00	40.0	99.4±2.1	0.0
2	1.00	60.0	99.7±0.2	0.0
3	5.00	40.0	83.1±2.6	5.5±0.7
4	5.00	60.0	58.0±0.6	14.3±0.6
5	0.17	50.0	100.2±1.2	0.0
6	5.83	50.0	44.1±0.1	15.3±1.0
7	3.00	35.9	100.1±0.9	0.16±0.0
8	3.00	64.1	95.0±0.3	2.9±0.4
9	3.00	50.0	99.3±2.5	0.8±0.3
10	3.00	50.0	100.6±1.1	0.3±0.1

ANOVA results showed that both water content in ethanol and reaction temperature had significant effect on biodiesel yield and triglyceride in biodiesel (p-value <0.05). The predicted equation of biodiesel yield and triglyceride in biodiesel were exhibited in equation 5.5 (R^2 $_{adjust}$ 0.94) and equation 5.6 (R^2 $_{adjust}$ 0.97), respectively. These predicted models were passed the lack of fit, thus they were used to generate the contour plot of predicted biodiesel yield and triglyceride in biodiesel as shown in Figure 5.3.

$$Biodiesel\ Yield\ (\%) = 37.82 + 28.07X_1 - 3.46X_1^2 + 1.71X_2 - 0.01X_2^2 - 0.32X_1X_2 \quad \text{Equation 5.5}$$

$$Triglyceride\ in\ Biodiesel\ (\%\ wt) = 24.55 - 8.37X_1 + 0.91X_1^2 - 0.77X_2 - 0.01X_2^2 - 0.11X_1X_2 \quad \text{Equation 5.6}$$

where X_1 was water content in ethanol (% vol) and X_2 was reaction temperature (°C).

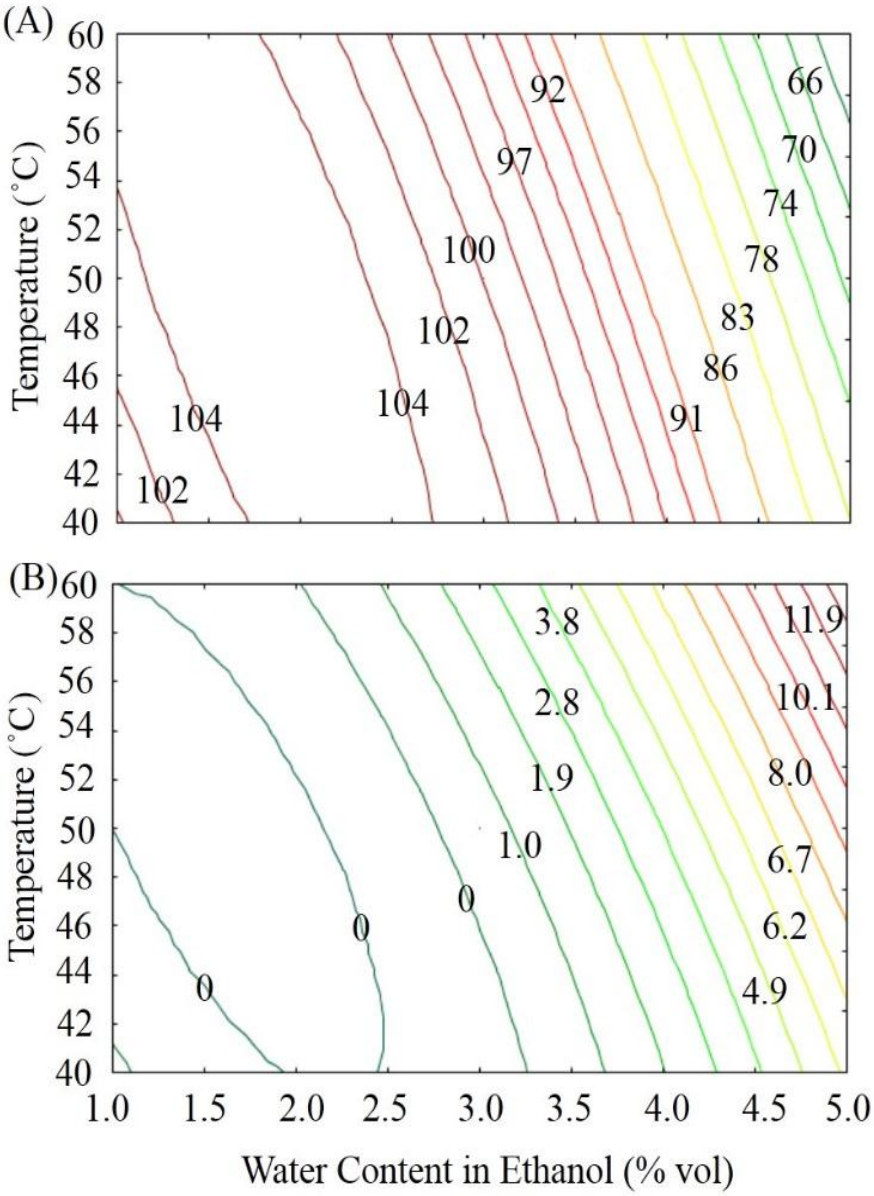


Figure 5.3 Biodiesel yield (A) and triglyceride in biodiesel (B) from aqueous ethanolysis *in-situ* TE at various temperature using Na (metal) concentration of 0.3% w/v, L-S ratio of 3 mL/g and shaking speed at 150 rpm for 60 min.

With presence of water in ethanol, elevating reaction temperature reduced biodiesel yield due to the soap formation from saponification reaction. Moreover, the soap formation is sodium salt of free fatty acid, thus generating soap reduces amount of Na (metal) in the reaction. This cause an incomplete TE reaction, therefore non-reacted triglyceride was found in biodiesel product (Figure 5.3B).

The saponification reaction could be prevented by reducing the oil solubility of water via lower the reaction temperature. The results in Figure 5.3 and HPLC-ELSD chromatograms (Figure 5.4) showed that less triglyceride in biodiesel was observed under the lower reaction temperature conditions.

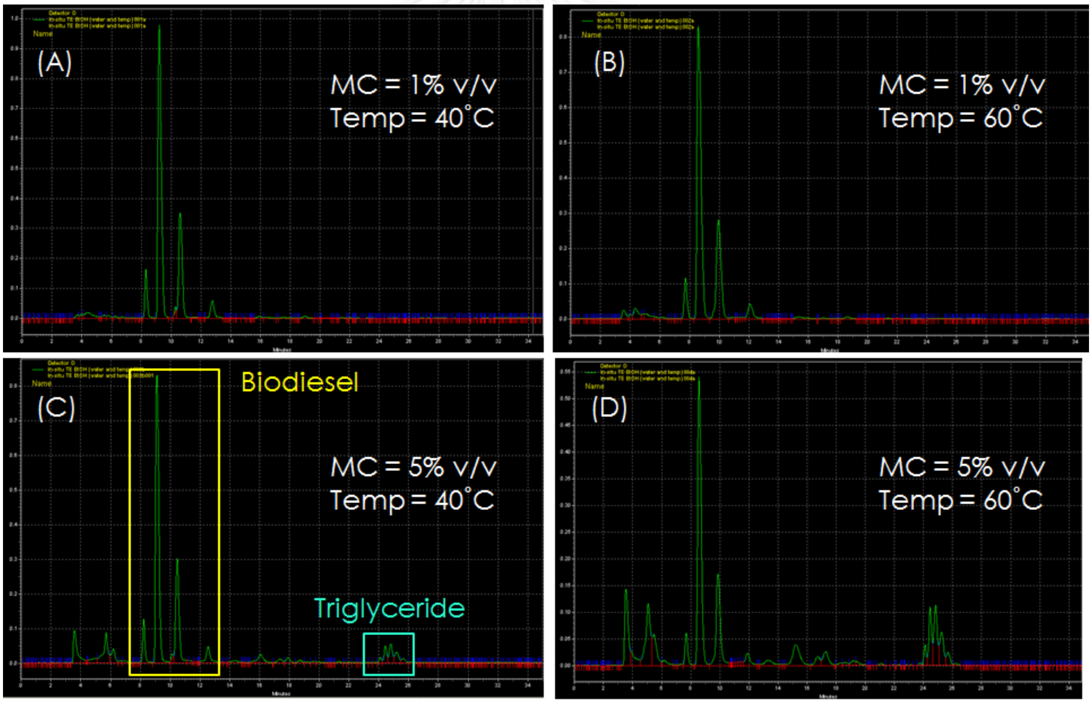


Figure 5.4 HPLC-ELSD chromatogram of biodiesel and triglyceride at (A) 1% MC with 40 °C, (B) 1% MC with 60 °C, (C) 5% MC with 40 °C and (D) 5% MC with 60 °C. MC was moisture content in ethanol.

At reaction temperature of 40 °C, the maximum water content in ethanol which still maintain 100% biodiesel yield was 3.3% vol. This finding could save the alcohol cost in the process approximately 56% based on ethanol price in the market as presented in Table 5.6. However, the aqueous ethanol 96.7% cost was far more expensive than methanol. To further reduce ethanol cost, the water content in aqueous ethanol 95% could be reduced by using the water adsorbent rather than blending it with absolute ethanol. Another solution was to use denature ethanol (i.e. mixture of alcohol with absence of ethanol tax).

Table 5.6 Prices of alcohol (OCT 9, 2015)

Alcohol	Moisture Content (% vol)	Supplier	USD/L
Aqueous ethanol 95%	5.0	VWR	5.28
Ethanol ACS grade	<0.05	VWR	80.14
Methanol ACS grade	<0.05	VWR	5.18
Aqueous ethanol 96.7%	3.3	-	35.24
Denatured ethanol - Ethanol 90% - Methanol 5% - Isopropanol 5%	<0.5	VWR	12.56

5.4. Conclusion

Applying acetone to enhance oil solubility of methanol had both positive and negative effect on biodiesel yield depended on proportion of acetone used in the process. The negative effect of using acetone as co-solvent was observed when using high proportion of acetone due to the catalyst precipitation (i.e. Na (metal) cannot be dissolved in acetone). The catalyst precipitation induced the soap formation during the *in-situ* TE process, resulting in lowering biodiesel yield. This effect was increased once applying the higher level of L-S ratio since the higher concentration driving force increased the triglyceride extraction from the oilseed. The extracted triglyceride then freely reacted with the precipitated catalyst and produced soap formation. Nevertheless, 100% of biodiesel could be achieved under the optimal conditions of acetone proportion, and it required less alcohol, lower temperature and shorter reaction time than those of using neat methanol in *in-situ* TE.

The biodiesel yield of *in-situ* TE could be enhanced by using higher hydrophobic alcohol such as ethanol and isopropanol in place of methanol. According to optimal conditions of using different alcohol in *in-situ* TE, applying ethanol and isopropanol required less alcohol loading and Na (metal) concentration than those of methanol. Also, 100% of biodiesel yield could be achieved within 60 min at 30 °C. Both ethanol and isopropanol are renewable and relatively safer than methanol. However, these alcohols are more expensive and might require much more energy for alcohol recovery after the *in-situ* TE process. To reduce the cost of ethanol, the aqueous ethanol was used in *in-situ* TE. The water content in ethanol at 3.3% vol was the maximum level that still maintain the 100% biodiesel yield of ethanolysis *in-situ* TE at 40 °C. This can reduce cost of ethanol approximately 56%. Moreover, the maximum water content in ethanol could be increased once the lower reaction temperature was applied.

CHAPTER 6

DEVELOPING AND SCALING-UP *IN-SITU* TRANSESTERIFICATION FOR BIODIESEL PRODUCTION USING SPENT COFFEE GROUND FROM INSTANT COFFEE INDUSTRY

According to previous study on *in-situ* TE for soybean biodiesel production using several alcohol systems and their optimal conditions, methanol and NaOH were used in this part due to the economic perspective. The main objective of this part was to develop on-site SCG biodiesel production for an instant coffee plant. Several challenges had to be overcome.

The oil of SCG has a very high acid value due to the roasting and coffee brewing process, which potentially reduces the biodiesel yield when using NaOH as catalyst. Therefore, a pretreatment step to reduce the acid value of SCG oil (deacidification) is very important. Another problem of SCG biodiesel from instant coffee production is that the coarse, roasted matrix of the SCG particles makes a portion of the oil inaccessible for *in-situ* TE; a better understanding is needed of the effect of SCG particle size on the performance of the process at different process temperatures. One last concern is the lack of understanding of the kinetics of the *in-situ* TE process when used with SCG. After calculating the kinetic parameters of developed process, the process was scaled up to 4 kg SCG per batch, and finally the biodiesel quality was evaluated according to the ASTM biodiesel standard.

6.1. Characterization of SCG

More than 50% of dried SCG was initially retained by the US sieve size No. 12 (i.e., particle size >1.68 mm), and this portion was ground and again sieved to five fractions; however only four fractions were used as described in chapter 3. The mass distribution of the four SCG size ranges used in this study, their oil contents, and acid values were shown in Table 6.1. The whole SCG had an oil content of 18.8% wt of dried SCG weight. The oil content was within the range reported in the literatures (Kondamudi et al., 2008; Haile et al., 2013; Kwon et al., 2013). The fatty acid profile of the extracted oil was comprised of more than 40% of polyunsaturated fatty acids, mainly linoleic acid (42%) as shown in Figure 6.1. Saturated fatty acids were also detected at high percentage (44%) where palmitic acid (34%) was dominant. The fatty acid profile of SCG oil in this study was similar to that reported in the literature (Vardon et al., 2013).

Table 6.1 Size distribution after grinding of SCG and DSCG with the corresponding oil content and acid value.

Size range	% wt	Oil content (% wt)		Acid value (mg KOH g/oil)	
		SCG	DSCG	SCG	DSCG
1.19 - 1.68 mm	37.98	18.8±0.5	17.2±0.9	5.93±0.02	0.59±0.12
1.00 - 1.19 mm	16.75		17.8±0.9		0.56±0.18
0.42 - 1.00 mm	41.22		19.4±0.5		0.56±0.09
<0.42 mm	4.05		20.4±0.5		0.56±0.23

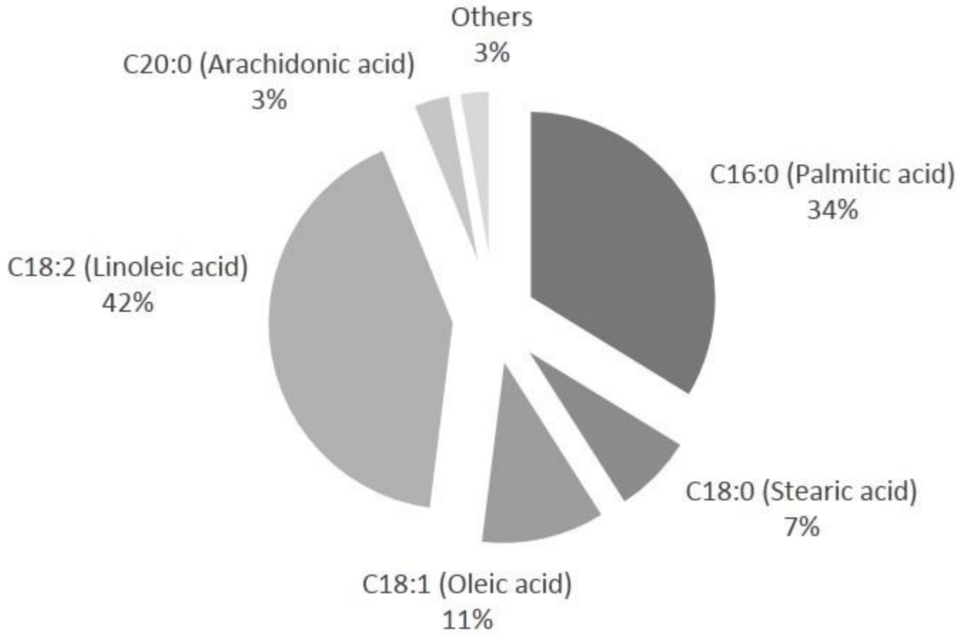


Figure 6.1 Fatty acid profile of SCG oil used in this study.

The initial acid value of SCG used in this study was high (5.93 mg KOH g/oil), which indicates a high content of FFA. SCG oil typically has a high acid value of 7 – 13 mg KOH g/oil (Al-Hamamre et al., 2012; Vardon et al., 2013) due to the roasting of coffee beans and coffee brewing process, type of coffee bean (i.e., arabica and robusta), moist storage conditions after the brewing process, and SCG drying process. The SCG in this study had a slightly lower acid value than the literature values. This was probably because drying commenced within one day after the coffee brewing process; and the dried SCG was then stored at 4 °C. Nevertheless, the acid value of SCG oil in this study still exceeded the recommended level for the *in-situ* TE process. The FFA potentially neutralizes alkaline catalysts, resulting in lower biodiesel yields during TE and greater refining loss. The development of deacidification process was required.

6.2. Optimizing Deacidification Process

A methanol washing technique was applied as the pretreatment process to reduce the high acid value in SCG. The experiment was designed according to CCRD with two factors, which were L-S ratio and washing temperature using SCG particle size 0.25 - 0.42 mm. The design of experiment and total oil content in DSCG and its acid value were presented in Table 6.2. The purpose of this part was to reduce the acid value of SCG to <1 mg KOH/g (i.e. recommended level for TE reaction) with high oil content in DSCG after the process. Statistica program version 10 was applied to optimize the condition, and analyze ANOVA and regression coefficient. The ANOVA results of total oil content in DSCG and acid value were shown in Table A9 and A10, respectively.

Table 6.2 Experimental design to optimize deacidification process with respect to total oil in DSCG and its acid value.

Treatment	L-S ratio (mL/g)	Temperature (°C)	Oil in DSCG (% wt)	Acid Value (mg KOH/g)
1	2.00	35.00	19.1±0.4	1.17±0.02
2	2.00	55.00	21.3±1.7	1.35±0.13
3	4.00	35.00	15.9±0.4	0.70±0.04
4	4.00	55.00	15.4±0.5	0.76±0.05
5	1.59	45.00	19.5±0.5	1.88±0.07
6	4.41	45.00	14.6±1.3	0.73±0.05
7	3.00	30.86	19.0±0.8	0.90±0.07
8	3.00	59.14	16.8±1.0	1.08±0.05
9	3.00	45.00	19.0±0.8	0.92±0.04
10	3.00	45.00	20.8±0.6	0.95±0.01

The ANOVA results showed that there was no significant effect of L-S ratio and temperature on oil content in DSCG after the process (Table A10), but the L-S ratio did significantly affect the acid value of DSCG oil (Table A9). These were due to the dramatic difference in solubility of triglyceride and FFA in methanol. The low triglyceride solubility in methanol resulting in an insignificant effect on oil content in DSCG (i.e. low oil extraction performance). Meanwhile FFA has high solubility in methanol which potentially extract FFA from SCG, leading to lower acid value.

The total oil in DSCG and its acid value from the experiments and deacidification conditions were used to develop quadratic models for predicting total oil in DSCG (adjusted R^2 0.833) and its acid value (adjusted R^2 0.746) as presented in equation 6.1 and 6.2, respectively. Both equations passed the lack-of-fit test, and were used to plot the contour graphs, which presented the oil content in DSCG and its acid value in Figure 6.2A and 6.2B, respectively.

$$Total\ oil\ (\%) = -12.51 + 9.07X_1 - 1.34X_1^2 + 1.00X_2 - 0.01X_2^2 - 0.07X_1X_2 \quad \textbf{Equation 6.1}$$

$$Acid\ value\ (mgKOH\ /g) = 2.42 - 1.09X_1 + 0.15X_1^2 + 0.02X_2 \quad \textbf{Equation 6.2}$$

where X_1 was L-S ratio (mL/g) and X_2 was washing temperature (°C).

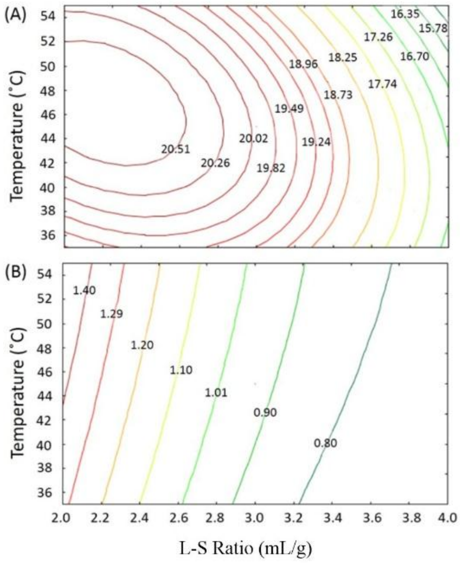


Figure 6.2 Effect of L-S ratio and process temperature on oil content in DSCG (A) and its acid value (B) after deacidification, using SCG size range of 0.25 – 0.42 mm, extraction time 60 min with 15.7 rad/s mixing speed.

Several conditions provided higher oil content than the initial oil content of SCG. This was due to the solvent property of methanol. Methanol selectively extracts hydrophilic compounds in SCG (FFA, phospholipid and antioxidant), but slightly extracts hydrophobic compounds (triglyceride). Reducing proportion of hydrophilic compounds increases the proportion of triglyceride, resulting in higher total oil content in DSCG.

The predicted results from Figure 6.2B presented the conditions that could reduce the acid value of SCG particle in the size range 0.25 – 0.42 mm to the recommended level < 1 mg KOH/g. However, a preliminary experiment showed that there was an effect of SCG size range on the performance of the deacidification process (data not shown). Using coarse size of SCG tended to reduce the performance of FFA removal by methanol washing. To meet the recommended level of acid value, the L-S ratio was set at 3 mL/g with 45 °C of extraction temperature. The resulting oil content and acid value in DSCG of four different size ranges showed that the acid value was reduced to below the recommended level with acceptable oil content (Table 6.1). These deacidification conditions were selected and used in the *in-situ* TE process.

6.3. Optimizing *In-situ* TE of SCG Biodiesel Production

The SCG from instant coffee process had a wide range of particle size as presented in Table 6.1. To compensate this effect, elevating temperature was evaluated its effect on biodiesel at four particle size of DSCG after deacidification process. The full factorial design of two factors with four levels was used in this section, and the biodiesel yields of each treatment and ANOVA result were shown in Figure 6.3 and Table A11, respectively.

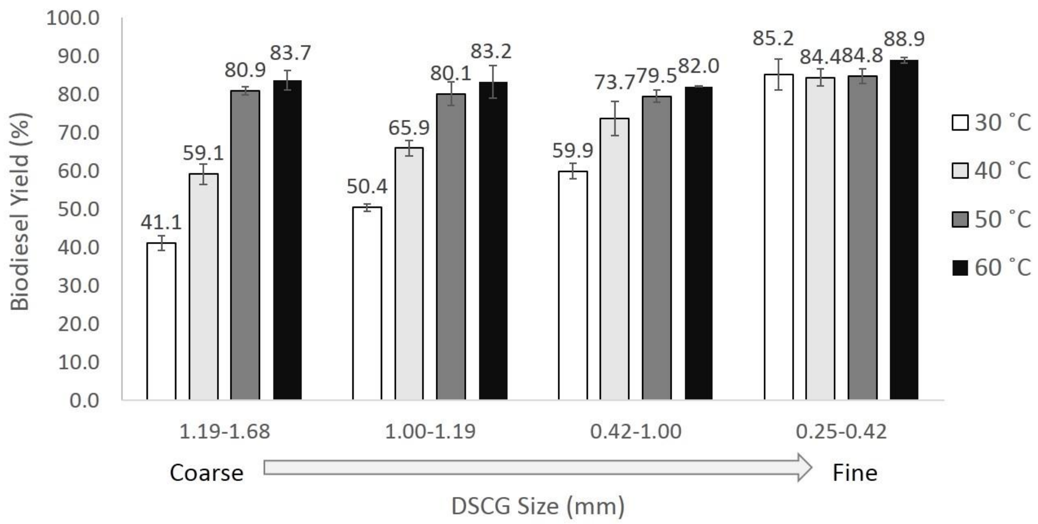


Figure 6.3 Effect of DSCG particle size and reaction temperature on biodiesel yield of *in-situ* TE process using L-S ratio 3 mL/g, NaOH concentration of 0.9% w/v and 15.7 rad/s of mixing speed for 3 h.

Reducing the DSCG particle size increased the biodiesel yield, as shown in Figure 6.3. The highest mean biodiesel yield of 85.8% (averaged for all reaction temperatures) was achieved using the finest DSCG particle size <0.42 mm. The benefit of finer particles was expected based on research on the effects of particle size on oil extraction from the oil-rich platelets by Boucher et al. (Boucher et al., 1942). They found that the particle thickness of oil bearing materials played an important role in the oil extraction rate and yield. This phenomenon is incorporated into industrial processes for the extraction of vegetable oil from oilseeds, and seeds such as soybean and canola are routinely processed into flakes having a width <0.4 mm before solvent extraction (Singh et al., 1999). However, roasted particles such as DSCG are not amenable to flaking. Only 4% of DSCG mass was <0.42 mm shown in Table 6.1. An additional energy-intensive grinding step would be required to reduce all of the SCG to this size, thus increasing the investment and operation costs of this process. Very fine particles are also more difficult to separate from solvent after extraction.

The effect of particle size on biodiesel yield was eliminated through use of elevated reaction temperature (Figure 6.3). The biodiesel yield for the coarsest size range increased from 41.1% to 83.7% when the DSCG temperature increased from 30 to 60 ˚C, as shown in Figure 6.3. As the particle size decreased, the difference in biodiesel yield among the different DSCG reaction temperatures decreased. The biodiesel yield was similar across reaction temperatures for the finest size range. The effects on biodiesel yield of DSCG size range, reaction temperature and interaction between them were significant (*p*-value < 0.001) as shown in Table 6.5. The reaction temperature effect is due to the improved oil solubility and viscosity of biodiesel and methanol. At higher temperatures, the viscosity of methanol is lower, leading to an improved diffusion coefficient of the process (Boucher et al., 1942). Methanol with catalyst can penetrate faster and deeper into the DSCG matrix, resulting in more rapid, complete conversion of oil to biodiesel. Moreover, the high temperature improves the biodiesel reaction rate because it is a slightly endothermic reaction.

Although a reaction temperature of 60 ˚C provided a slightly higher biodiesel yield than 50 ˚C, the benefit was not great. The weighted average yield was 2.8% higher at 60 ˚C. The pilot scale reactor lacked a reflux condenser, thus a reaction temperature of 50 ˚C was used in subsequent experiments. Also, all DSCG fractions were combined in subsequent experiments.

6.4. Kinetic Parameters of Developed *In-situ* TE Using Whole DSCG at 50 ˚C

Biodiesel was prepared from whole DSCG for 11 reaction times from 0.01 to 3 h, to evaluate the effect of reaction time on total glyceride content and biodiesel yield (Figure 6.4). All refined biodiesel samples had a total glyceride content of <0.05% by mass, including samples obtained after just 0.01 and 0.02 h, indicating very rapid and complete conversion of extracted glycerides to biodiesel. These findings agreed well with the results from Zakaria and Harvey (2014). They also stated that under NaOH concentration >0.08 M in methanol, the reactive extraction rate of *in-situ* TE is controlled by extraction phenomena. The NaOH concentration in this study (0.225 M) was sufficient, thus the kinetic model of solvent oil extraction equation 3.1 proposed by Meziane and Kadi (2008) could be applied in this study.

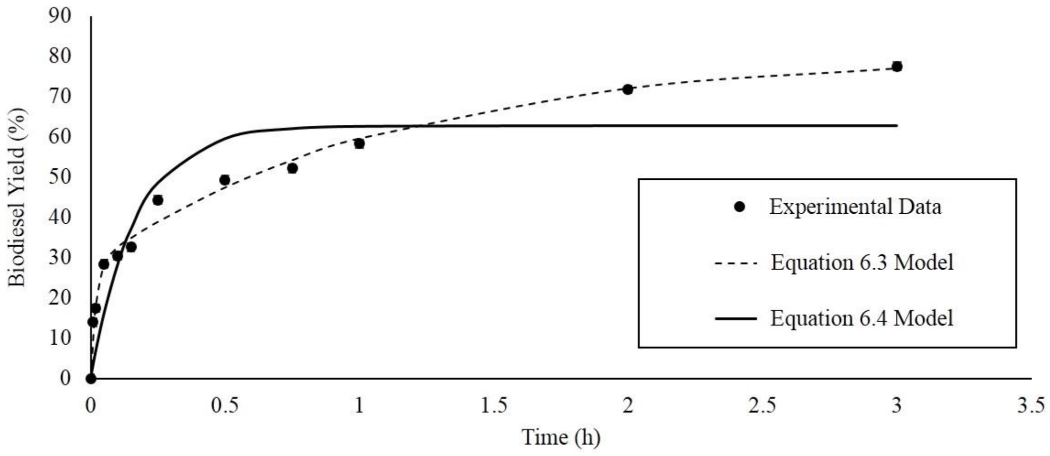


Figure 6.4 Biodiesel yield of 11 time points during *in-situ* TE process using whole DSCG, L-S ratio at 3 mL/g, NaOH concentration of 0.9% w/v, mixing speed of 15.7 rad/s and reaction temperature 50 °C.

As shown in Figure 6.4, the biodiesel yield increased rapidly with time in the first 0.05 h of the process, and then asymptotically approached the equilibrium yield. These data suggested that this process could be best described by the kinetic model in equation 3.1, based on the premise that two simultaneous processes occur during *in-situ* TE: (1) a rapid washing process to extract and transesterify oil located on or very near the surface of DSCG, and (2) a slower diffusion-limited process to extract and transesterify oil entrapped within the DSCG matrix.

The kinetic parameters in equation 3.1 were determined by minimization of the total chi-squared value. The resulting model is presented in equation 6.3, as well as in Figure 6.4 (dashed line). A very good fit between the model and the experimental data can be observed, with an adjusted R^2 of 0.98. After a 3 h reaction time, the predicted biodiesel yield was 76.9%, which was slightly lower than that of the experimental yield of 77.5±2.6%.

$$Y_t = 28.2(1 - e^{-51.6t}) + 51.9\,(1 - e^{-0.92t}) \qquad \textbf{Equation 6.3}$$

The experimental data was also fitted to a simple, commonly-used first-order kinetic model which used only two kinetic parameters (single rate constant and equilibrium yield) as shown in equation 6.4 and Figure 6.4 (solid line); its adjusted R^2 was only 0.74, which was far smaller than that achieved using the model shown by equation 6.3, and Figure 6.4 (dashed line) displays the inadequacy of the simple model.

$$Y_t = 62.8(1 - e^{-5.9t}) \qquad \textbf{Equation 6.4}$$

This confirmed the value of modeling this *in-situ* TE process as a combination of rapid wash and slower diffusion processes. According to equation 6.3, the diffusion process dominated the performance of *in-situ* TE due to the high Y_D and low k_D values. To improve the diffusion step, the use of a finer particle size, elevated temperature and pressure and multiple extraction steps could be considered in a future study, but would result in a more costly process.

6.5. Pilot-scale *In-situ* TE and Biodiesel Quality

The biodiesel quality and yields for four small scale (30 g) and two pilot-scale (4 kg) batches of SCG production are presented in Table 6.3. This SCG biodiesel had a dark, transparent appearance and strong roasted coffee odor. It met nearly all of the most recommended ASTM biodiesel standards in terms of moisture content, total glyceride, kinematic viscosity and OSI, as presented in Table 6.3. However, it had high cloud point, pour point and acid value. The average biodiesel yield for the two pilot-scale batches was 81.8±1.6%.

Table 6.3 Biodiesel yield and quality of *in-situ* TE SCG biodiesel at both small scale and pilot-scale, which were performed using whole DSCG at 50 °C for 3 h of reaction time.

Parameter	ASTM D6751	*In-situ* TE Process (Reactive Extraction)			Conventional Process (Oil Extraction + TE)	
		Small scale	Pilot Scale		Vardon et al. (2013)	Kondamudi et al. (2008)
			1	2		
Production scale (SCG mass)	-	30 g	4 kg	4 kg	No report	100 g
Moisture content (ppm)	500	247±23	204±6	227±4	632±2	No report
Total glycerol (% mass)	0.240 max	0.08±0.00	0.05±0.03	0.04±0.00	0.10	0.11
Kin. Visc. 40° C (mm^2/s)	1.9-6.0	4.18±0.03	3.81±0.08	4.33±0.05	5.19±0.00	5.84
Cloud point (°C)	Report	-	12.8±0.4	13.0±0	13.1±0.3	11.0
Pour point (°C)	Report	-	10.3±0.4	9.5±0.3	13.0±0.0	2.0
Oxidative stability index (h)	3.0 min	-	4.9±0.5	8.8±0.1	0.2±0.0	3.0
DPPH assay EC$_{50}$ (mg/mL)	-	-	3.3	2.6	No report	No report
Acid value (mg KOH/g)	0.50 max	1.40±0.18	5.10±0.05	2.68±0.11	0.11±0.01	0.35
Biodiesel Yield (% mass based on oil in DSCG)	-	77.5±2.6	80.7	83.0	96*	100*

* Biodiesel yield was calculated based on amount of DSCG oil used in TE step.

6.5.1. Biodiesel quality

6.5.1.1. Moisture content, total glyceride and kinematic viscosity

In terms of moisture content, our biodiesels at both scales met the ASTM standard. This low level of moisture content ensures the quality of biodiesel during storage. Although, the SCG biodiesel of Vardon et al. (2013) slightly exceeded the ASTM standard, the standard is readily met through gentle vacuum drying. In the cases of total glycerol and kinematic viscosity, all SCG biodiesels produced via *in-situ* TE were within the ASTM standard; and these values agree well with those reported in Vardon et al. (2013) and Kondamudi et al. (2008). These results show adequate conversion of glycerides to methyl esters.

6.5.1.2. Cloud point and pour point

SCG biodiesel has poor cold flow properties, as reported by others. The cloud and pour point exceeded 9.5 °C. This is due to the high content of saturated fatty acid (>44%, as shown in Figure 6.1). Therefore, the neat SCG biodiesel (B100) is not suitable for use in cold climates. However, blending the SCG biodiesel with petroleum diesel lessens this problem. The cloud point and pour point of B5 SCG diesel fuel (95:5 volume ratio of petroleum diesel to SCG biodiesel) were -13.2 and -22.7 °C, respectively (Vardon et al., 2013). Biodiesel is already commonly blended with ultralow sulfur petroleum diesel (ULSD) at 2 – 5% by volume to improve the poor lubricity of the ULSD fuel.

6.5.1.3. Oxidative stability index (OSI)

The OSI value indicates the resistance of biodiesel to oxidation during storage. Biodiesel with a low OSI value (<3 h) is especially sensitive to runaway oxidative reactions which generate sediment, and increase acid value and viscosity during storage. SCG biodiesel may be at somewhat high risk for low OSI values due to its high proportion of linoleic acid (C18:2) as shown in Figure 6.1, in combination with the coffee bean roasting step. The two double bonds in linoleic acid make it sensitive to oxidation. Soybean oil is also comprised of >50% of linoleic acid (Figure 2.4); however, the OSI value is far better than that of SCG biodiesel produced via conventional biodiesel production. Thus, roasting and brewing coffee steps might be major impacted on the low OSI value of SCG biodiesel.

Since SCG oil is sensitive to oxidation, the process temperature and duration of operation time potentially affect the OSI value of SCG biodiesel. In the conventional processes reported in the literature, multiple steps involving heating are required to produce biodiesel including (1) up to several hours of oil extraction using n-hexane followed by desolventizing; (2) esterification to reduce the high acid value of SCG oil; and (3) transesterification followed by biodiesel purification.

An important advantage of our *in-situ* process is fewer steps involving heating, thereby explaining our superior OSI values for the pilot-scale process reported in Table 6.3. Our second pilot-scale batch had an even much higher OSI value than the first batch; this was because of the gentler methanol recovery step used in the second batch, as described below in section 6.5.1.4. Hence, a rapid process with low process temperature is the key to achieving high OSI and overall quality of SCG biodiesel. This also accounts for the higher OSI achieved by Kondamudi et al. (2008) compared to Vardon et al. (2013), though both groups applied n-hexane oil extraction in their process.

In addition to our gentler process conditions, the superior OSI value of the biodiesel in this work might also be due to natural antioxidants in our SCG biodiesel, especially chlorogenic acid and melanoidins. These antioxidant compounds are known to be present in SCG. They are hydrophilic and readily extracted by methanol (Suárez-Quiroz et al., 2014; Naidu et al., 2008) during the *in-situ* TE process, and partially retained during the refining steps. This hypothesis is partly supported by the appearance and odor of *in situ* SCG biodiesel reported above, which were much more pronounced compared to SCG biodiesel produced using the hexane-extraction step. Moreover, after exposed with 110 °C and presence of air from OSI analysis, the dark color of SCG biodiesel turned to yellow (Figure 6.5), thus this dark compound in our SCG biodiesel might be an antioxidant.

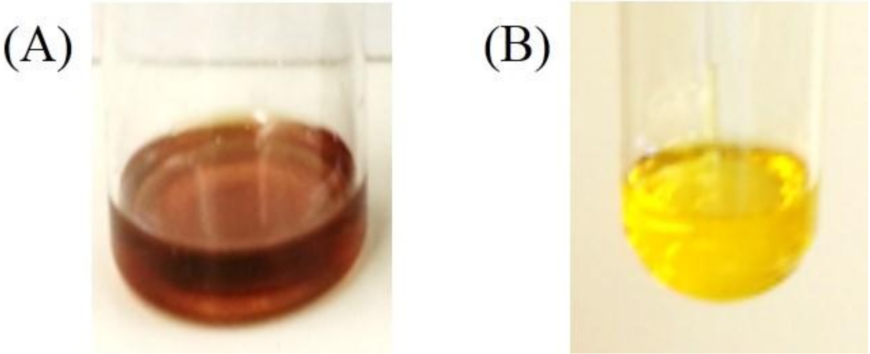


Figure 6.5 SCG biodiesel before (A) and after (B) applying 110 °C and air purging during OSI analysis.

To further support this hypothesis, antioxidant activity was determined using the DPPH assay and compared with that of conventional SCG biodiesel. The conventional SCG biodiesel was synthesized in our lab following the procedure described by Vardon et al. (2013). The average EC_{50} value of SCG biodiesel from the *in situ* TE process was 10 times lower than that of our conventional SCG biodiesel, thereby indicating a higher antioxidant activity in the former samples.

Blending our SCG biodiesel with petroleum diesel might present a problem, because chlorogenic acid and melanoidins are not very soluble in hydrophobic liquids (i.e., diesel). These natural antioxidants might precipitate and cause clogging of diesel fuel filters. To determine whether this is a problem, 2% by volume of biodiesel was blended with petroleum diesel at 30 °C. The blended fuel was transparent with no visible precipitate. However, after centrifugation for 20 min at 3,000 g, a small amount of dark brown wax-like precipitate was observed (Figure 6.6). Hence, biodiesel-diesel blend should be pre-filtered or treated with a co-solvent to avoid plugging the vehicle fuel filter with this precipitate. Nevertheless, the presence of natural antioxidant in *in-situ* SCG biodiesel still provides a benefit of preserving the biodiesel quality during storage before blending with petroleum diesel.

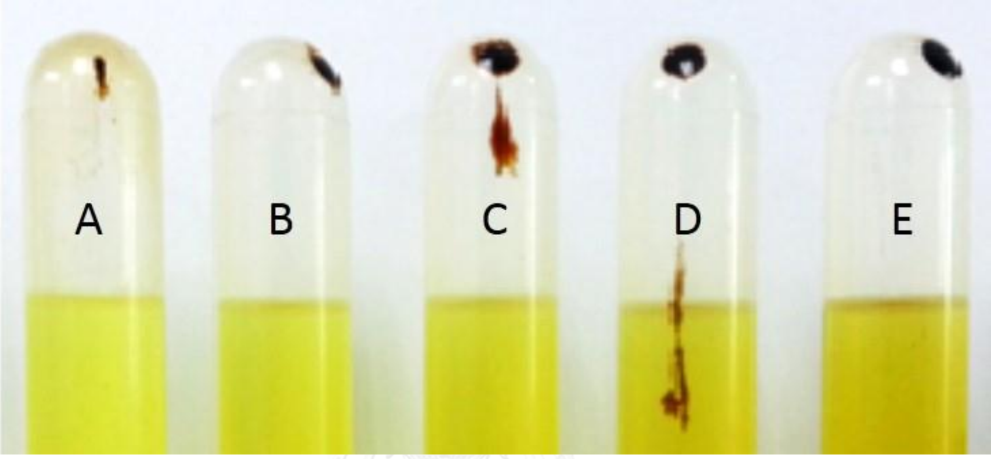


Figure 6.6 Blending SCG biodiesel with petroleum diesel at 2 (A), 4 (B), 6 (C), 8 (D) and 10% vol (E) after applying centrifuge force at 3,000 g for 20 min.

6.5.1.4. Acid value

SCG biodiesel from *in-situ* TE had a higher acid value compared to biodiesel from the conventional process and did not pass the ASTM standard. Biodiesel possessing high acid value potentially causes corrosion in diesel engine and fuel sediment, resulting in filter plugging. However, the SCG biodiesel produced by Vardon et al. (2013) and Kondamudi et al. (2008) was well within the ASTM standard for acid value.

Subsequent to the pilot-scale tests, we suspected our high acid value was due to excess H_2SO_4 used to neutralize alkaline catalyst, and/or the long time involved in the methanol removal step. To test the first of these hypotheses, filtrate from the reaction mixture after 3 h was titrated; the titration showed the residual alkaline catalyst to be only 10% of that added at the start of the process. We had assumed that all of the catalyst would be present in this filtrate, and calculated the amount of H_2SO_4 based on that assumption. The high acid value in our SCG biodiesel was likely due in part to excess unreacted H_2SO_4; therefore, the amount of H_2SO_4 for neutralization can be correspondingly less. To confirm this assumption and develop a solution, a crude biodiesel in n-hexane was produced as outlined in section 3.5.2; it was first washed with acidic water (0.051 M H_2SO_4) following by twice DI water washing, in accordance with Haagenson et al. (2010), in place of the previous neutralization step with H_2SO_4 immediately after *in situ* TE (Figure 3.4). This change greatly improved the acid value of the refined biodiesel to 0.28 ± 0.18 mg KOH/g which thus passed the ASTM standard, and reduced the need for H_2SO_4. Therefore, this method will be adopted in our future SCG biodiesel production process.

The second of the above hypotheses was suggested upon comparing the acid value for the small-scale biodiesel with that of the pilot scale; the acid value of the pilot-scale biodiesel was significantly higher (Table 6.3). The notable difference between these two different process scales was the methanol evaporation step. In contrast to the small scale process, the methanol evaporation step at the first pilot-scale batch had a very long operation time (8 h) as well as a high heating-mantle temperature (90 °C). Under these conditions, as well as excess H_2SO_4 and the presence of water from the neutralization of alkaline catalyst, hydrolysis of biodiesel likely occurred, resulting in FFA generation. To reduce the high acid value of the biodiesel in the second pilot-scale batch, the methanol evaporation step was changed to 10 h at 75 °C; the acid value of the second batch was reduced by almost 50% compared to the first batch (Table 6.3). However, it still exceeded the ASTM standard and the value achieved at the small scale. As with OSI, a rapid process with low process temperature is the key to achieving high quality (low acid value) SCG biodiesel. Hence, applying vacuum and increasing the heat transfer surface area during evaporation would significantly reduce the evaporation temperature and duration of the methanol evaporation. These approaches will be incorporated into the future SCG biodiesel production process.

6.5.2. Performance of pilot-scale *in-situ* transesterification

The average biodiesel yield for the two pilot-scale batches was 81.8±1.6% and yields were not significantly different from small scale (*p*-value >0.05). The residual oil content in defatted SCG after the pilot-scale *in-situ* TE process was 3.8±0.1% mass of defatted SCG which accounts for <18% of unrealized biodiesel yield. The remainder of the unrealized biodiesel yield was attributed to the process separation and refining steps. This residual oil can be a good source of energy if the defatted SCG is used to produce heat energy. The heat content of defatted SCG in this study was 20.3 MJ/kg.

It was not possible to directly compare biodiesel yields with those from the conventional methods in Table 6.3, because their yields were based solely on the TE step. The residual oil of the defatted SCG in the two other studies was probably underestimated, because that material was not reground and re-extracted, as was done in our study. Also, the esterification step in Vardon et al. (2013) and related steps to neutralize and remove acid catalyst, and the alkali refining step used in Kondamudi et al. (2008) should result in yield reductions not accounted for in the biodiesel yields reported in those studies. Therefore, the actual biodiesel yield of the *in situ* TE process may be similar to the conventional processes. The simpler and gentler *in-situ* TE process may be more suitable for an on-site biodiesel production plant when taking into account the reduction in process steps and time, and also additional benefit in obtaining high OSI values. The *in-situ* TE process could be scaled up to a much larger scale while achieving yield and quality similar to the pilot scale.

6.6. Conclusions

Simply washing SCG with methanol could reduce high acid value, and still preserve the oil in DSCG after deacidification process. Then, an *in situ* TE (reactive extraction) process was first tested at small scale (30 g SCG per batch) and then successfully scaled up to 4 kg SCG per batch. SCG particle size reduction and an elevated reaction temperature improved the SCG biodiesel yield of the *in-situ* TE process; a biodiesel yield of > 80% was achieved within 3 h at 50 °C using whole DSCG. The SCG biodiesel from our *in-situ* TE process had a superior OSI value compared to biodiesel obtained from the conventional process (separate steps for solvent oil extraction and biodiesel synthesis), due to co-extraction of natural antioxidants with gentler and simpler process conditions. The kinetic parameters found in this study may be applied to achieve further process improvements in the future. Such an *in-situ* TE process can then be applied at instant coffee plants for production of biodiesel as a valuable byproduct and to improve waste management. The SCG biodiesel could be both produced and used locally, thereby enhancing benefits to the environment and contributing to the economy of the community. The defatted SCG residue from the process could be a source of other valuable bio-products.

CHAPTER 7

ENERGY USAGE AND ENVIRONMENTAL IMPACTS ASSESSMENT OF SCG BIODIESEL PRODUCTION

The developed on-site SCG biodiesel production using *in-situ* TE process from previous chapter was extrapolated to industrial level for applying as an on-site SCG biodiesel production at an instant coffee plant. In order to determine the performance, LCA was introduced to evaluate the energy usage and environmental impact of this approach, and compared the results with those of conventional process which the SCG has to be transported to central facilities for oil extraction by n-hexane, oil purification, and biodiesel synthesis and purification steps. Therefore, applying an on-site SCG biodiesel production from our study could reduce the cost of transportation and eliminate the use of hazardous n-hexane and complexity of oil extraction and purification steps. The hotspot of each approach was identified to further process development. Several suggestion approaches were proposed at the end of this chapter. Finally, the distance of transportation and fuel consumption rate of vehicle were used as sensitivity factors to determine the energy usage of conventional process, whose results could be used as the guideline to select the suitable transportation system or apply decentralization process instead.

7.1. Goal and Scope

The study is accomplished with the four steps according to the International Standardization Organization (ISO) standard (ISO 14040, 2006; ISO 14044, 2006). The goal of the study was to compare the energy usage and environmental impacts of biodiesel production using SCG waste from instant coffee plant. Two different scenarios of biodiesel production approaches were studied in this study:

- Conventional process: a conventional process that the SCG had to be transported from instant coffee plant to central facility where the SCG oil was extracted by n-hexane extraction before converted to biodiesel using 2-step TE.
- On-site *in-situ* TE: our developed reactive extraction process, a combination process of oil extraction and biodiesel synthesis, using sodium methoxide solution as biodiesel reagent and solvent. No SCG transportation since this process was installed at an on-site of instant coffee plant.

The system boundary was “Gate-to-Gate” included SCG pretreatment process at instant coffee plant to SCG biodiesel product. Thus, the cultivation of the coffee bean, coffee roasting and brewing process were not included in this study as well as the use of SCG biodiesel as biofuel. One kg of SCG biodiesel was used as a functional unit.

7.2. Life Cycle Inventory

Data for inventory analysis were collected from several literatures. The overview of two different approaches for SCG biodiesel production was exhibited in Figure 3.2.The sub-processes of each approach were described in section 7.2.1 and 7.2.2 for conventional and decentralization process, respectively.

7.2.1. Conventional process (Scenario I)

The SCG was transported to central biodiesel production facilities involving n-hexane oil extraction process, biodiesel synthesis and purification. After that, the SCG biodiesel was transported back and used in the instant coffee process. It should be noted that the secondary data from several literatures were used in this section including process conditions along with performance and energy usage of the instruments such as pump, distillation, mixer motor and etc. The detail of each step was presented below. The overall process of SCG oil extraction and biodiesel synthesis was illustrated in Figure 7.1. The inventory analysis of this approach was shown in Table 7.1.

Table 7.1 Inventory process of a conventional process for producing one kg SCG biodiesel.

Drying & Transportation					
Feed		Product (kg)		Waste (kg)	
SCG (kg)	7.81	15% MC SCG	6.43	Water	1.38
Heat (MJ)	5.86				
Diesel (MJ)	1.25				
Oil Crushing					
Feed		Product (kg)		Waste (kg)	
15% MC SCG (kg)	6.43	Defatted SCG	4.48	Water	Not report
Heat (MJ)	3.94	SCG Oil	0.99	Hexane	1.11E-02
Electricity (KWh)	0.21				
2-step Transesterification					
Feed		Product (kg)		Waste (kg)	
SCG Oil (kg)	0.99	Biodiesel	1.00	Na_3PO_4	4.46E-03
Methanol (kg)	0.21	Glycerol	0.09	H_2SO_4 + Glycerol + Methanol	8.59E-02
H_2SO_4 (kg)	9.13E-03			Water + Methanol	3.83E-02
NaOH (kg)	3.26E-03				
H_3PO_4 (kg)	2.67E-03				
Heat (MJ)	3.55				
Electricity (KWh)	2.66E-03				
Transportation (Biodiesel)					
Feed		Product (kg)		Waste (kg)	
Diesel (MJ)	1.25				

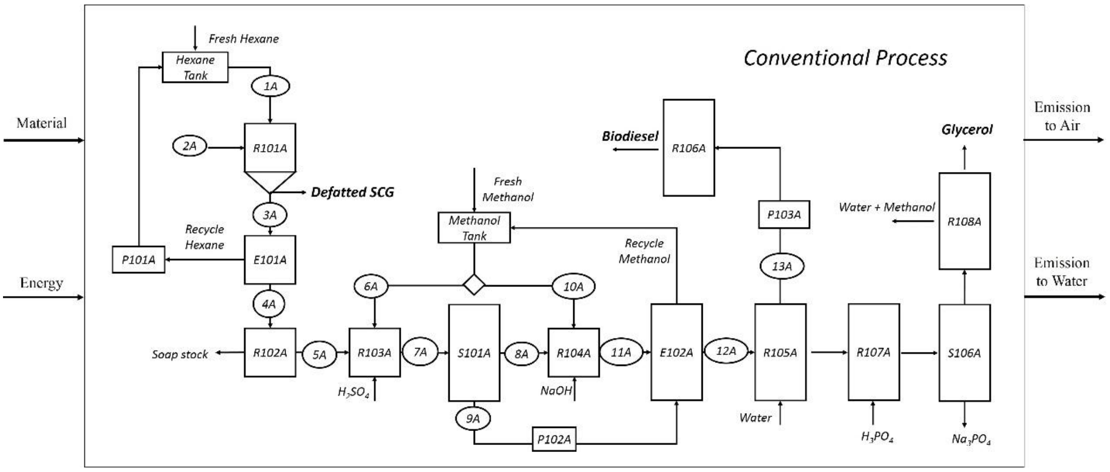


Figure 7.1 System boundary of conventional SCG biodiesel production at central facilities.

7.2.1.1. Drying and SCG transportation

To reduce MC of SCG from 30 to 15% mass, 1.13 kg of water had to be removed before SCG transportation by dryer using natural gas as the energy source. The 15% MC SCG (6.43 kg) was then transport to central facility for SCG oil extraction and biodiesel production using a truck 28t ETH model (40% load) as carrier. The distance between instant coffee process and the central facility was fixed at 35 km (i.e., 70 km for round trip).

7.2.1.2. SCG oil extraction

There has not been report of SCG oil extraction process conditions by n-hexane in the industry scale. With similar oil content of 18.9%, thus the process conditions of n-hexane soybean oil extraction were applied in this section. The conditions and performances of oilseed pretreatment (additional drying and grinding), oil extraction using n-hexane (*R101A* in Figure 7.1) and oil purification (*R102A* in Figure 7.1) in this section were followed Pradhan et al. (2011). Given 100% performance of n-hexane oil extraction, all of SCG oil was extracted. Then, the SCG oil was used in 2-step TE for biodiesel production. There was an assumption that in every tons of input oilseed, approximately 11.1 mL of n-hexane was lost during the oil extraction process (Hass et al., 2004).

7.2.1.3. 2-step TE

Due to the high acid value from free fatty acid (FFA) in SCG oil (*5A* in Figure 7.1), the pre-treatment step comprised of esterification using H_2SO_4 as catalyst was required before TE step using NaOH as catalyst. With similar acid value, the process conditions and performance of esterification and TE using wasted cooking oil as biodiesel feedstock by Varanda et al. (2011) were applied in this section. In esterification (*R103A* in Figure 7.1), the methanol-to-oil molar ratio of 6 with 0.9% of H_2SO_4 at 70 °C and 400 kPa were applied. All FFA in SCG oil was converted to biodiesel. Then, the pretreated SCG oil (*8A* in Figure 7.1) was converted to biodiesel using methanol-to-oil molar ratio of 6 and 1% of NaOH catalyst at 60 °C and 400 kPa.

7.2.1.4. Methanol recovery

The crude biodiesel with glycerol (*9A* and *11A* in Figure 7.1) was sent to a multi-stage vacuum distillation for methanol recovery (*E102A* in Figure 7.1). The conditions and performance of the process were used those described in Varanda et al. (2011). The four stages and a reflux ratio of 2 were applied to ensure the high quality of the product. The methanol was recycled to the process.

7.2.1.5. Biodiesel water washing

The liquid-liquid extraction technique using 21 °C water (*R105A* in Figure 7.1) was used to separate biodiesel from glycerol, methanol and catalyst (*12A* in Figure 7.1). The process conditions and performance were used those present in Varanda et al. (2011).

7.2.1.6. Biodiesel and glycerol purification

In order to achieve high quality of biodiesel (purity >99.6%) and glycerol (purity >93%), the four stage vacuum distillation with 2 of reflux ratio (*R106A* and *R108A* in Figure 7.1) was applied (Varanda et al., 2011).

7.2.1.7. Removal of catalyst

The NaOH catalyst was neutralized by H_3PO_4, and formed Na_3PO_4 salt as the waste.

7.2.2. On-site *in-situ* TE process (Scenario II)

The SCG was used as the biodiesel feedstock in on-site biodiesel production units at the instant coffee plant. Thus, there was no SCG and its biodiesel transportation. The conditions and performances of the processes were applied the results from our pilot scale operation (4 kg SCG/batch). The overall of biodiesel production process was illustrated in Figure 7.2. In cases of industrial instruments, we applied the specification and energy usage from Kaewcharoensombat et al. (2011). The inventory analysis of this process was shown in Table 7.2.

Table 7.2 Inventory process of an on-site *in-situ* TE process for producing one kg SCG biodiesel.

Drying and Grinding					
Feed		Product (kg)		Waste (kg)	
30% MC SCG (kg)	9.20	Dried SCG	6.44	Water	2.76
Heat (MJ)	11.73				
Electricity (KWh)	5.60E-04				
In-situ TE Process					
Feed		Product (kg)		Waste (kg)	
Dried SCG (kg)	6.44	Defatted SCG	5.46	Water + Glycerol	0.14
Methanol (kg)	30.55	Biodiesel	1.00	Methanol	6.37E-03
NaOH (kg)	0.20	Glycerol	9.14E-02	Coffee Soap	2.04E-02
H_2SO_4 (kg)	0.23	Na_2SO_4	0.35	Methanol Extracted	3.54E-02
Water (kg)	2.72E-02				
Heat (MJ)	62.06				
Electricity (KWh)	5.77E-02				

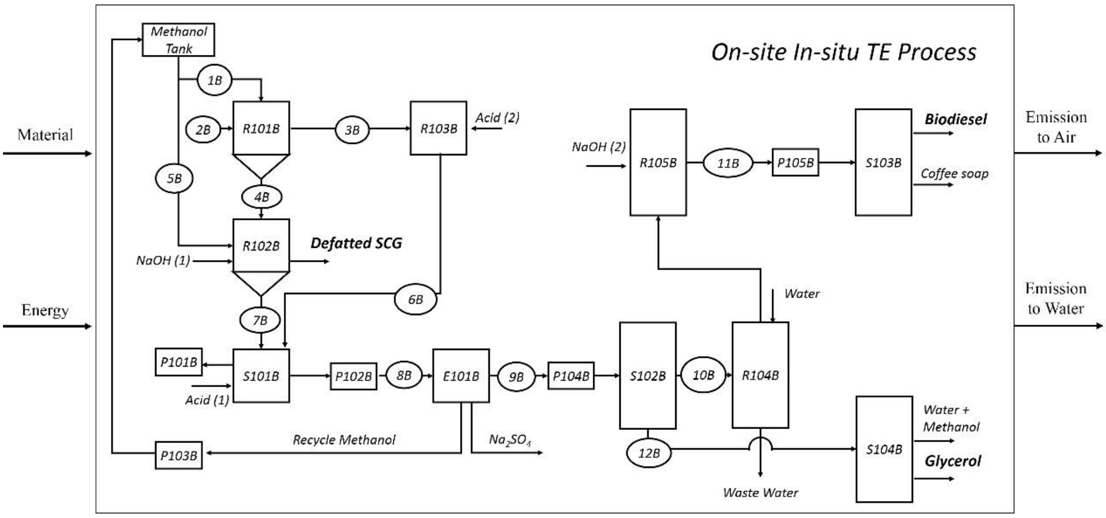


Figure 7.2 System boundary of an on-site *in-situ* TE for SCG biodiesel production process.

7.2.2.1. Drying and grinding

The sundried SCG with 30% MC was reduced its MC to < 1% MC using a same dryer mentioned in section 7.2.1.1 of conventional process. Approximately 2.76 kg of water had to be removed from SCG. The dried SCG (6.44 kg) was then ground by Micro-Max air swept fine grinder, model MM1600 (http://www.stedman-machine.com/micro-max-fine-grinders.html).

7.2.2.2. Deacidification

Due to the high acid value from FFA in SCG, the pretreatment which reduced the acid value was required. In this study, the solid-liquid extraction technique using methanol was applied. Methanol is very selective solvent; it can extract the FFA, but still preserves the quantity of oil (triglyceride) in SCG due to its hydrophilic property. The methanol-to-SCG ratio of 3 mL/g was applied with 300 rpm mixing speed at 45 °C for 1 h (*R101B* in Figure 7.2). Then, the slurry was filtrated through the metal sieve for separating deacidified SCG (DSCG; *4B* in Figure 7.2) from liquid fraction (methanol extracted; *3B* in Figure 7.2). The oil content in DSCG was 18.34% mass with acid value <0.5 mg KOH/g oil. The titration result of methanol extracted and molecular weight of triglyceride in SCG (858 g/mol) showed that approximately 0.16 kg of FFA was found in this methanol extracted.

7.2.2.3. Esterification

H_2SO_4 (*Acid (2)* in Figure 7.2) was directly added to methanol extracted (*3B* in Figure 7.2) at 1% w/w concentration as the acidic catalyst to convert all FFA to biodiesel. The process temperature was set at 50 °C for 0.5 h (*R103B* in Figure 7.2).

7.2.2.4. In-situ TE

The DSCG was treated with 0.9% w/v sodium methoxide solution, prepared by dissolving NaOH in methanol. This solution directly converts the triglyceride in DSCG to biodiesel and glycerol, which were able to be extracted by sodium methoxide solution. The sodium methoxide solution-to-DSCG was 3.5 mL/g at 50 ºC and mixing at 300 rpm for 3 h (*R102B* in Figure 7.2). Then, the solid fraction (defatted SCG) was separated from liquid fraction (diluted biodiesel and glycerol in sodium methoxide solution; *7B* in Figure 7.2) by metal sieve with vacuum pumpassisted (*P101B* in Figure 7.2).

7.2.2.5. Catalyst neutralization

The liquid fractions from esterification (*6B* in Figure 7.2) and *in-situ* TE (*7B* in Figure 7.2) were pooled together. Then, H_2SO_4 (*Acid (1)* in Figure 7.2) was added to neutralize the alkaline catalyst; and formed Na_2SO_4 salt as the by-product.

7.2.2.6. Methanol recovery

The methanol in neutralized liquid fraction (*8B* in Figure 7.2) was evaporated by a vacuum distillation column with 7 stages (*E101B* in Figure 7.2) as described in Kaewcharoensombat et al. (2011). Recycle methanol was send back to use in the process.

7.2.2.7. Biodiesel purification

The crude biodiesel and glycerol were separated by gravity tank (*S102B* in Figure 7.2). At the bottom of the tank, crude glycerol (*12B* in Figure 7.2) was sent to glycerol purification process; then the upper crude biodiesel was washed by DI water as described in Kaewcharoensombat et al. (2011). After biodiesel washing, the 10% w/v aqueous NaOH solution (*NaOH (2)* in Figure 7.2)) was added to biodiesel for reduced the acid value; and centrifuged to separate the soap from biodiesel using US centrifuge systems, model MAC 250 (*S103B*, Figure 7.2) (http://www.uscentrifuge.com/mac-250.php).

7.2.2.8. Glycerol purification

The bottom crude glycerol was purified to 93% using the multi-distillation column (*S104 B*, Figure 7.2) described in Kaewcharoensombat et al. (2011).

7.2.3. Allocation procedure

In spite of biodiesel, several valued by-products could be obtained during the process; for example: glycerol and defatted SCG. Therefore, the energy usage and environmental impacts of these by-product had to be acknowledged. In this work, the mass-based allocation method was applied to determine how the energy usage and environmental impacts were attributed among these products at different biodiesel production processes shown in Figure 7.3A and 7.3B.

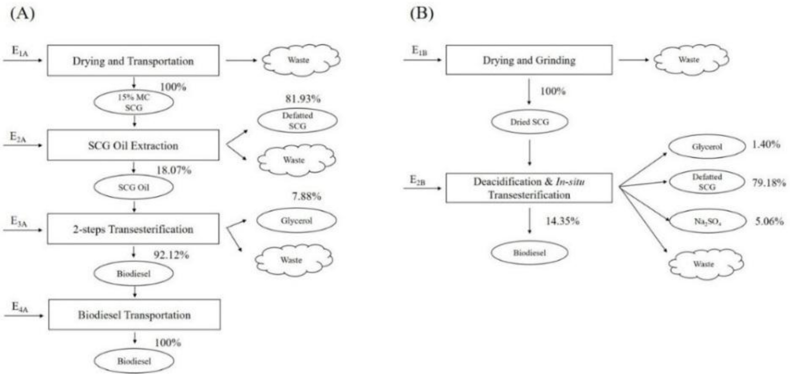


Figure 7.3 Mass-based allocation of biodiesel and its by-products from conventional (A) and on-site *in-situ* TE processes (B).

7.3. Life Cycle Impact Assessment

The IMPACT 2002+ methodology was used to quantify the impacts in the LCA with three parts: midpoint, endpoint and single score. It should be noted that the plant construction and waste treatment were not included in this study. Also, the environmental impacts from producing SCG was not included in this assessment; since it was considered as the waste from the instant coffee industry.

7.4. Sensitivity Analysis

The sub-objective of this study was to determine which situation would be suitable to produce SCG biodiesel via conventional or on-site *in-situ* TE process. Here in this part, the energy usage was used as the selecting criteria. In the real situation, the SCG source might be very disperse and significant effect on the energy usage in transportation section. Therefore, the distance of transportation between SCG source and central facilities for biodiesel production was considered to set up as sensitivity factor (60 – 240 km). In addition, the difference in diesel consumption of vehicle was also determined in the study (4 – 18 km/L). The results will provide the useful information which vehicle (i.e., truck and train) should be used for transportation according to its fuel consumption and distance.

7.5. Interpretation

7.5.1. Energy usage

The energy usage of conventional process was dramatically lower than that of on-site *in-situ* TE as shown in Figure 7.4. To improve the energy usage, the hotspot of the process had to be identified. For conventional process, the hotspot of the process was biodiesel synthesis & purification which was contributed to >50% of total energy usage or 3.27 MJ/kg biodiesel, followed by biodiesel transportation (19.3%), SCG drying (14.9%), oil crushing (12.0%), and SCG transportation (3.4%), as shown in Figure 7.4A. Unlike edible biodiesel feedstock (soybean, palm and canola), the defatted SCG could be used as energy source, and considered as a renewable energy due to its high heating value.

The result of analyzing heating value of defatted SCG was 20.3 MJ/kg; therefore, it can be estimated that we could obtained heat energy approximately 90 MJ from defatted SCG for every 1 kg of biodiesel product (Figure 7.3A). This energy alone was sufficient to support for the whole SCG biodiesel production in both conventional and on-site *in-situ* TE process. In a case of on-site *in-situ* TE; moreover, the excess energy could be used in the instant coffee process. This could increase the interest in installing an on-site SCG biodiesel production unit in the instant coffee plant.

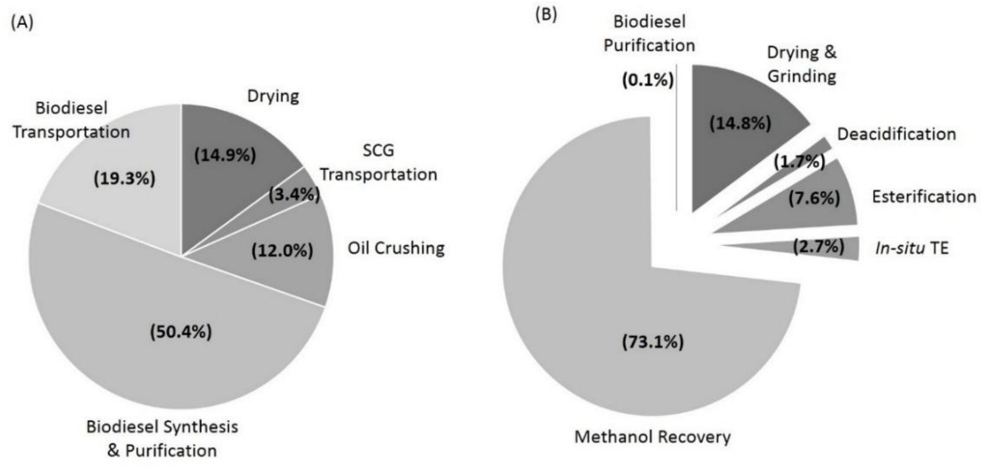


Figure 7.4 Percentage of energy usage of each sub-process in conventional (A) and on-site *in-situ* TE process (B). The energy usage of conventional and on-site *in-situ* TE process were 6.49 and 11.38 MJ/kg biodiesel, respectively.

In a case of on-site *in-situ* TE process (Figure 7.4B), the majority of energy usage was come from methanol recovery step, which was responsible for 73.1% of the total energy usage or 8.33 MJ/kg biodiesel, followed by drying & grinding (14.8%), esterification (7.6%), *In-situ* TE (2.7%), deacidification (1.7%), and biodiesel purification (0.1%). This was due to the large amount of methanol used in this process with 30.94 kg methanol for producing only one kg of SCG biodiesel.

Tremendous energy (i.e., process steam from natural gas) was required to separate the enormous amount of methanol from biodiesel. In order to solve this problem, we had to reduce the amount of methanol loading such as reusing methanol in deacidifcation and *in-situ* TE steps. Reusing methanol increases the concentration of biodiesel before methanol evaporation; therefore, greatly decrease the energy usage per kg of biodiesel product in methanol recovery step. Another solution was applying countercurrent extraction technique in place of simple batch extraction reactor. This option can accumulate the extracted biodiesel during the process and enhance the extraction performance due to improving in concentration driving force (Beckel et al., 1946). The future study on reusing methanol and/or countercurrent extraction for deacidifcation and *in-situ* TE steps would worth to be investigated.

The great advantage of on-site *in-situ* TE was the absence of feedstock and biodiesel transportation which consumed energy >20% in the conventional process (Figure 7.4). However, this energy usage would increase based on the distance of transportation route and also the fuel consumption rate of vehicle as shown in Figure 7.5. The result suggested that the on-site *in-situ* TE process was more desirable once the transportation distance was more than 180 km at fuel consumption rate of 6.38 km/L. If the transportation distance was >180 km, the combination of vehicle (i.e., truck, barge and rail) should be considered. The result in Figure 7.5 might be used as one of the proposed options to select the suitable combination of vehicle for feedstock and biodiesel transportation or applying on-site *in-situ* TE process instead.

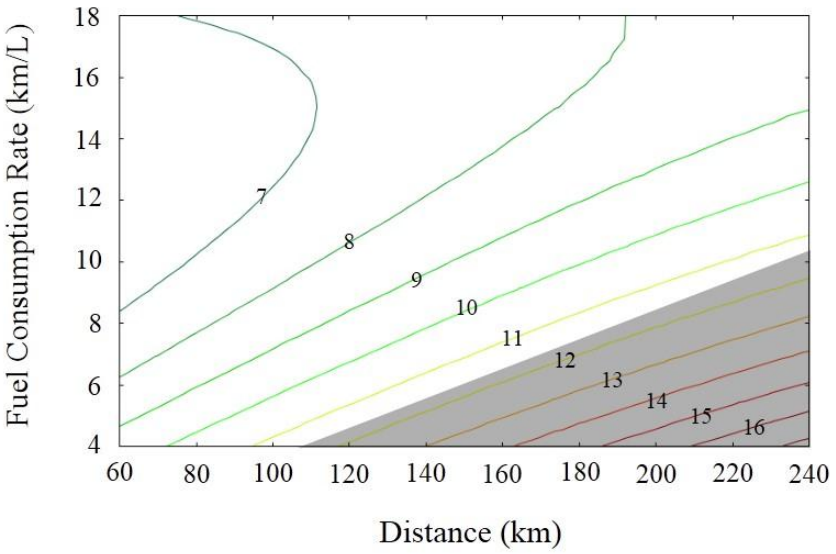


Figure 7.5 Energy usage (MJ/kg biodiesel) of conventional process at various distance of transportation and fuel consumption rate of vehicle; the shade area indicated the conditions which had energy usage higher than that of an on-site *in-situ* TE process (11.38 MJ/kg biodiesel).

7.5.2. Environmental impacts

The midpoint results suggested that producing SCG biodiesel via conventional process gave better environmental impacts than those of on-site *in-situ* TE process because of the tremendous energy used in methanol recovery step of on-site *in-situ* TE process (Figure 7.6). However, an on-site *in-situ* TE process provided lower environmental impacts in terms of respiratory organics and land occupation.

The extremely high impact of respiratory organs in conventional process was mainly come from n-hexane leaking during the process. Clean Air Act (1990) categorized n-hexane as a hazardous air pollutant. The amount of n-hexane leaking from the process in this work was come from the average of soybean oil n-hexane extraction plants in USA at the crushing capacity >2,400 ton soybean daily (Pradhan et al., 2011). The smaller n-hexane oil extraction plants might release more n-hexane than we used in this work. Therefore, this should be emphasis the advantage of using *in-situ* TE process which is n-hexane free process in our study.

An on-site *in-situ* TE process had slightly lower environmental impact on land occupation due to the absence of transportation (Figure 7.6). In IMPACT 2002+, the road construction is accounted in the analysis, so it includes the impact of the land used change due to the road construction. This impact could be increased based on the transportation distance between SCG source and central facilities.

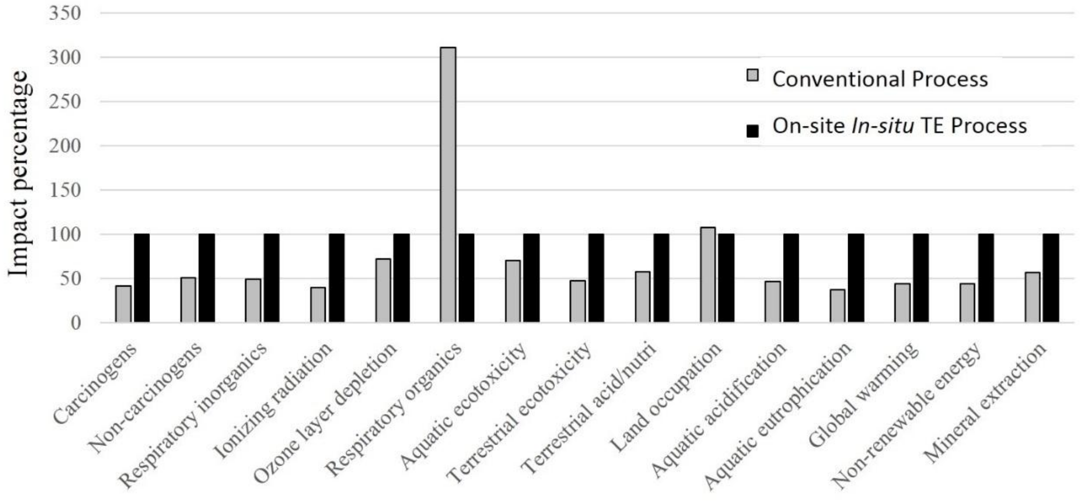


Figure 7.6 Relative environmental impacts of SCG biodiesel production using conventional and on-site *in-situ* TE process according to IMPACT 2002+ midpoint.

The fifteen environmental impact categories in Figure 7.6 (midpoint) was groped up into four main impacts (endpoint) according to IMPACT 2002+ (Figure 7.7). The results showed that producing SCG biodiesel using on-site *in-situ* TE process had higher environmental impacts than those of conventional process in terms of human health, ecosystem quality, climate change and resource. Similar result also reported by Nazir et al. (2012) who evaluated the environmental impacts of producing jatropha biodiesel via *in-situ* TE and the conventional process. However, our results provide far better environmental impacts compared to Nazir et al. (2012), since they did not allocate the environmental impact to the co-products (i.e. jatropha meal and glycerol). Also, their boundary did not include the feedstock and biodiesel transportation, which was the advantage of on-site in-situ TE process.

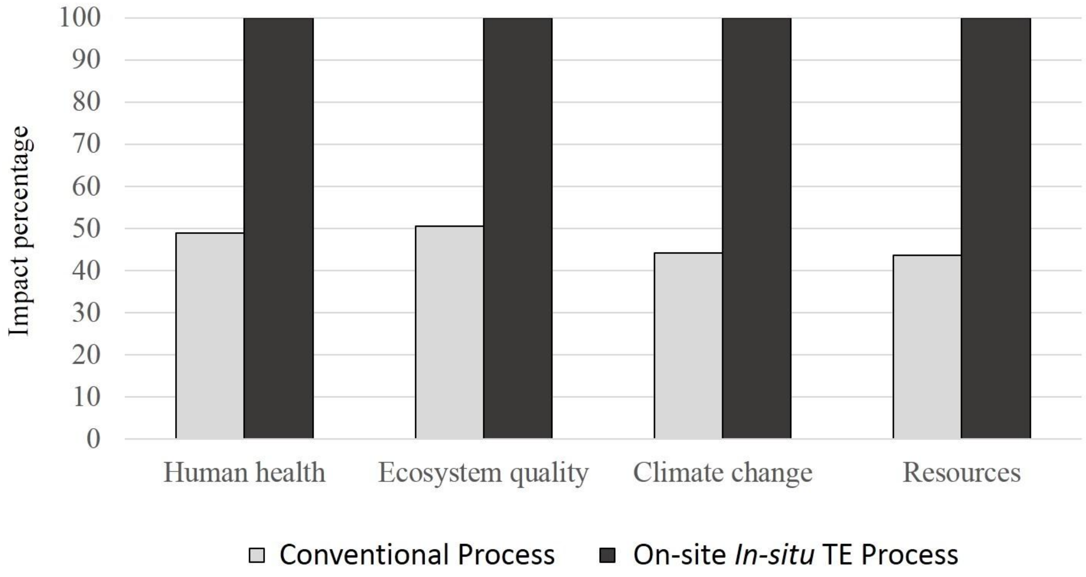


Figure 7.7 Relative environmental impacts of SCG biodiesel production using conventional and on-site *in-situ* TE process according to IMPACT 2002+ endpoint.

The hotspot of each environmental impacts (endpoint) were identified based on form of energy and leaking of the solvent (Figure 7.8). The results showed that steam was the major source of environmental impacts in both conventional and on-site in-situ TE processes. Especially in on-site *in-situ* TE, using steam was contributed to more than 90% of all environmental impacts. In this study, the steam was produced from natural gas. To reduce the environmental impact of steam, the alternative energy source should be considered.

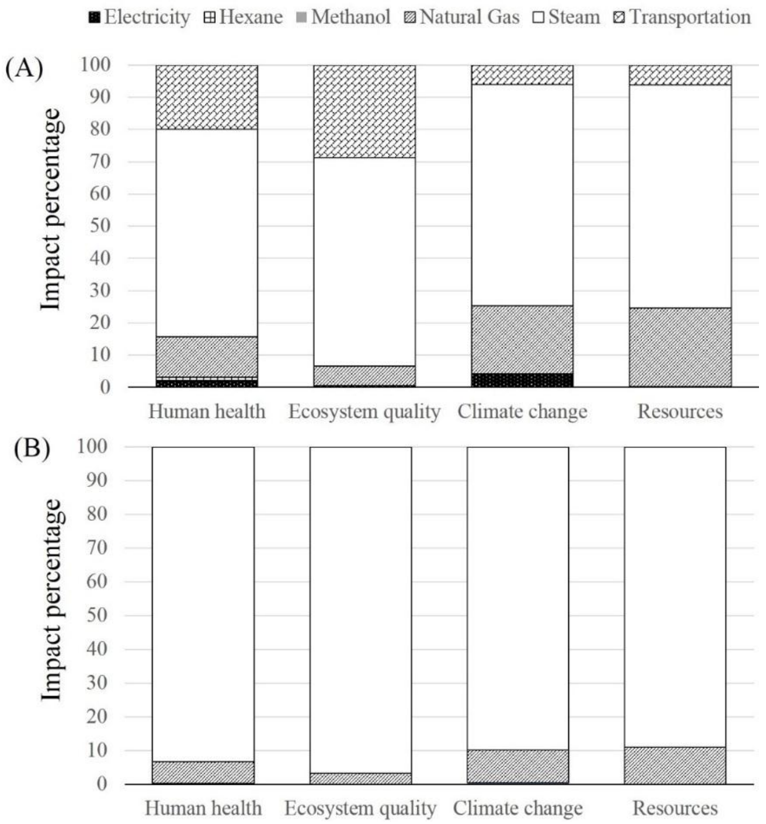


Figure 7.8 Relative environmental impacts of conventional (A) and on-site *in-situ* TE (B) process based on energy form and solvent leaking from the system, according to IMPACT 2002+ endpoint.

According to the analyzing of energy usage (Figure 7.4B) and endpoint results (Figure 7.8B), methanol recovery step which used steam as the heat source was the hotspot of the on-site *in-situ* TE process. The results of single score was able to confirm this hotspot, since the climate change and resource depletion were the main concerned for biodiesel production (Figure 7.9). These two impacts were principally affected by energy demanding process, which was methanol recovery step that consume up to 90% of the total energy usage. As mention previously, the defatted SCG could be used as energy source to produce steam in the process, which could reduce the environmental impacts in terms of climate change and resource depletion. Moreover, reusing methanol and applying countercurrent extraction would greatly decrease the energy usage per kg of biodiesel product in methanol recovery step.

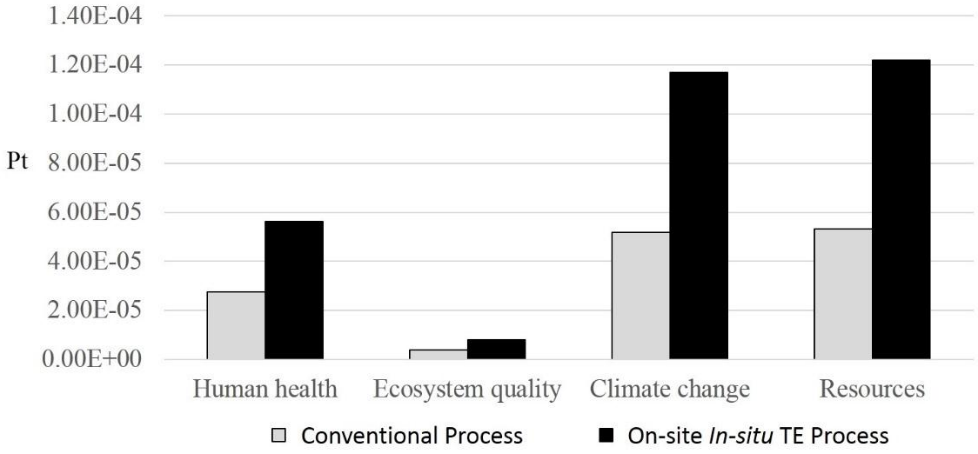


Figure 7.9 Comparison of the environmental impacts of producing SCG biodiesel via conventional and on-site *in-situ* TE process, according to IMPACT 2002+ single score. Units correspond to points (Pt).

7.6 Conclusions

Producing SCG biodiesel via conventional process had lower energy usage and environmental impacts than those of on-site *in-situ* TE. Nevertheless, an on-site *in-situ* TE process showed better environmental impacts in terms of respiratory organ and land occupation due to absence of hazardous n-hexane and transportation, respectively. Methanol recovery step was identified as the hotspot of an on-site *in-situ* TE process; since it consumed >90% of total energy usage and used steam produced from natural gas as the heat source. Reusing methanol with catalyst, applying countercurrent extraction and utilizing the heat waste from instant coffee industry were proposed to reduce the energy usage and environmental impacts of on-site *in-situ* TE process. Also, the defatted SCG by-product could be used as energy source to generate heat and steam for the whole process which reduced the negative impacts on climate change and resource depletion. Finally, sensitivity analysis of energy usage on transportation distance and fuel consumption rate suggested that the on-site *in-situ* TE process was more favorable once the transportation distance was >180 km with 7 km/L of fuel consumption rate.

CHAPTER 8

CONCLUSION AND FUTURE WORK

8.1. Conclusion

In-situ TE is a new approach to produce biodiesel directly from oilseed. The complexity of vegetable oil extraction using hazardous hexane and oil purification are eliminated. Such a simple approach was more suitable for small biodiesel production scale. Therefore, it could set up at an on-site of biodiesel feedstock source such as at the remoted area and in the industrial process (i.e. instant coffee plant in this study). This can reduce the cost of transportation and environmental burdens compared to the conventional process. In addition, the bio-active compounds such as antioxidant can be co-extracted from this process, which might increase the value of this process.

In this study, several parameters related to *in-situ* TE (i.e. reactive extraction) were evaluated their effect on the triglyceride extraction and biodiesel yield. The L-S ratio (i.e. alcohol loading), catalyst concentration and acetone proportion (i.e. hydrophobicity of alcohol system) were the three most important factors. Thus, they were applied to optimize the *in-situ* TE process using four difference alcohol systems, which were methanol, ethanol, isopropanol and methanol with acetone as co-solvent. The results showed that the more hydrophobicity of alcohol, the higher biodiesel yield was observed. Also, applying the stronger hydrophobic alcohol could reduce the alcohol loading and catalyst requirement, lower the reaction temperature and shorten the operation time. In several conditions, the soap formation was generated due to the promoting saponification reaction. Presence of water and applying exceed optimum catalyst concentration level were responsible for this problem. These negatives effect could be prevented or reduced by keeping water content in oilseed and alcohol as low as possible. However, operating the *in-situ* TE process at low temperature could inhibit the saponification as shown in the results of using aqueous ethanol in *in-situ* TE.

The characterization of biodiesel feedstock should be considered before using in the *in-situ* TE process. This issue is very important especially once the non-edible feedstocks are applied. The SCG waste from instant coffee process used in this study contained high water content (75% wt), high acid value (>5 mg KOH/g) and coarse particle size. The pretreatment processes, such as drying and grinding, were required. Simply washing SCG by methanol could reduce acid value in SCG to suitable level (<1 mg KOH/g). The optimal conditions of this deacidifcation was also investigated and applied as the pretreatment step. Elevating reaction temperature could reduce the negative effect of coarse particle size of SCG. The developed process of SCG biodiesel production was scaled up to 4 kg SCG loading per batch. The SCG biodiesel produced from *in-situ* TE process had superior in OSI value due to the co-extracted natural antioxidant in SCG. However, our SCG biodiesel had high acid value and did not pass the biodiesel standard. This might be due to the long methanol recovery step.

Once compare the energy usage and environmental impacts between developed process (on-site *in-situ* TE) and conventional approach at an industrial level, the LCA results indicated that producing SCG biodiesel via conventional process was better than on-site *in-situ* TE. Nevertheless, an on-site *in-situ* TE process showed better environmental impacts in terms of respiratory organ and land occupation due to absence of hazardous n-hexane and transportation, respectively. Methanol recovery step was identified as the hotspot of on-site *in-situ* TE process; since it consumed >90% of total energy usage and used steam produced from natural gas as the heat source. The sensitivity analysis of energy usage on transportation distance and fuel consumption rate suggested that the on-site in-situ TE process was more favorable once the transportation distance was >180 km with 7 km/L of fuel consumption rate. This information could be used as the guideline to select the suitable combination of vehicle for feedstock and biodiesel transportation or applying on-site *in-situ* TE process instead.

8.2. Recommendation for Future Study

Future study of *in-situ* TE should be indicated the biodiesel feedstock characteristics (i.e. oil content, acid value, moisture content, particle size and fatty acid profile), especially in the waste from other industries. The robustness and versatile of *in-situ* TE on different feedstocks or mixed should be investigated. The pretreatment process, which conditions the feedstock to be suitable for *in-situ* TE process, should be developed. Technology or approach, which reduces the alcohol and catalyst requirement, should be investigated such as reusing catalyzed alcohol and applying countercurrent extraction reactor (Figure C7). The co-benefit of applying *in-situ* TE process as an on-site biodiesel production should be further evaluated, for example integration of heat waste and recovery of bio-active compounds which are very valuable. The subsequence process, which further utilizes defatted oilseed, should be considered, including bioethanol, bio-composite material, bio-char, fertilizer and bioplastic. Such processes could be integrated and developed into the bio-refinery process. Finally, a cost analysis of *in-situ* TE process should be considered in the future.

In a case of SCG, the source of SCG from coffee shop would be very interesting. The keys of success are the suitable logistic system and collaboration with coffee shop, especially the franchise coffee shop such as Amazon®, Inthanin® and Starbuck®. The LCA would reveal the possibility to produce SCG biodiesel nationwide in Thailand, liked the success one “Bio-bean Ltd” in England (http://www.bio-bean.com/).

REFERENCES

Acheampong, K., Dicks, M. R., and Adam, B. D. "The Impact of Biofuel Mandates and Switchgrass Production on Hay Market." Southern Agricultural Economics Association Annual Meeting. Corpus Christi, Texas, USA, 2011.

Al-Hamamre, Z., Foerster, S., Hartmann, F., Kroger, M., and Kaltschmitt, M. Oil Extracted from Spent Coffee Grounds as a Renewable Source for Fatty Acid Methyl Ester Manufacturing. J. Fuel 96 (2012): 70-76.

Alternative Energy and Efficiency Information Center. Energy in Thailand : Facts & Figures Q1 - Q3 / 2013. 5 September 2014 2013. Department of Alternative Development and Efficiency. Available: http://www.dede.go.th/dede/images/stories/stat_dede/factsFigures_2013/q3_2013_re.pdf. 5 September 2014.

American Society for Testing and Materials. "Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels." ASTM D6751-09. West Conshohocken: ASTM, 2009.

Anastopoulous, G., Zannikou, Y., Stournas, S., and Kalligeros, S. Transesterification of Vegetable Oils with Ethanol and Characterization of the Key Fuel Properties of Ethyl Esters. Journal of Energies 2 (2009): 362-376.

Bala, B. K. Studies on Biodiesels from Transformation of Vegetable Oils for Diesel Engines. Journal of Energy Education Science and Technology 15 (2005): 1-43.

Beach, M., and Schumacher, L. "Lubricity of Biodiesel Blend." On Campus Undergraduate Research Internship Poster Session. Columbia, MO: University of Missouri, 2004.

Beckel, A. C., Belter, P. A., and Smith, A. K. Laboratory Study of Continuous Vegetable Oil Extraction: Countercurrent Extractor, Rissing-Film Evaporator, and Oil Stripper. Ind Eng Chem Res 18,1 (1946): 56-58.

Berchmans, H. J., and Hirata, S. Biodiesel Production from Crude *Jatropha Curcas* L. Seed Oil with a High Content of Free Fatty Acids. Bioresource Technology 99 (2008): 1716-1721.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., and Escaleira, L. A. Response Surface Methodology (Rsm) as a Tool for Optimization in Analytical Chemistry. Journal of Talanta 76 (2008): 965-977.

Booth, E. J. Extraction and Refining. In F. D. Gunstone (eds.), Rapeseed and Canola Oil: Production, Processing, Properties and Uses, pp.17-36. Florida: CRC Press, 2004.

Boucher, D. F., Barier, J. C., and Osborn, J. O. Extraction of Oil from a Porous Solid. Trans Am Inst Chem Eng 38 (1942): 967-993.

Çağlar, E. "Biodiesel Production Using Co-Solvent." European Congress of Chemical Engineering (ECCE-6). Copenhagen, 2007.

Carels, N. *Jatropha Curcas*: A Review. Advances in Botanical Research 50 (2009): 39-86.

Chisti, Y. Biodiesel from Microalgae. Journal of Biotechnology Advances 25 (2007): 294-306.

Choi, O. K., Song, J. S., Cha, D. K., and Lee, J. W. Biodiesel Production from Wet Municipal Sludge: Evaluation of in Situ Transesterification Using Xylene as a Cosolvent. Bioresour Technol 166,8 (2014): 51-56.

Cui, J., Lapan, H., Moschini, G. C., and Cooper, J. "Welfare Impacts of Alternative Biofuel and Energy Policies." Agricultural & Applied Economics Association's 2011 AAEA, CAES & WAEA Joint Annual Meeting. Denver, Colorado, USA, 2010.

Dantas, T. N. d. C., Silva, A. C. d., and Neto, A. A. D. New Microemulsion Systems Using Diesel and Vegetable Oils. Fuel 80 (2000): 75-81.

Demirbas, A. New Liquid Biofuels from Vegetable Oils Via Catalytic Pyrolysis. Journal of Energy Education Science and Technology 21 (2008): 1-59.

Demirbas, A., and Demirbas, M. F. Importance of Algae Oil as a Source of Biodiesel. Energy Conversion and Management In Press (2010).

Dewick, P. M. Essentials of Organic Chemistry: For Students of Pharmacy, Medicinal, Chemistry and Biological Chemistry. Wast Sussex, England: John Wiley & Sons Ltd, 2006.

Do, L. D., and Sabatini, D. A. Aqueous Extended-Surfactant Based Method for Vegetable Oil Extraction: Proof of Concept. Journal of American Oil Chemists' Society 87 (2010): 1211-1220.

Erhan, S. Z. Industrial Uses of Vegetable Oils. Champaign, IL: AOCS Press, 2005.

Freedman, B., Pryde, E. H., and Mounts, T. L. Variables Affecting the Yields of Fatty Esters from Transesterified Vegetable Oils. J Am Oil Chem Soc 61,10 (1984): 1638-1643.

Georgogianni, K. G., Kontominas, M. G., Pomonis, P. J., Avlonitis, D., and Gergis, V. Alkaline Conventional and in Situ Transesterification of Cottonseed Oil for the Production of Biodiesel. Energy Fuels 22 (2008): 2110-2115.

Go, A. W., Sutanto, S., Ong, L. K., and Tran-Nguyen, P. L. Developments in in-Situ (Trans) Esterification for Biodiesel Production: A Critical Review. Renew Sust Energ Rev 60 (2016): 284-305.

Go, H., Jiang, Y., Zhou, I., and Gao, J. Reactive Extraction and in Situ Self-Catalysted Methanolysis of Germinated Oilseed for Biodiesel Production. Energy Environ Sci 4,4 (2011): 1337-1344.

Gui, M. M., Lee, K. T., and Bhatia, S. Feasibility of Edible Oil Vs. Waste Edible Oil as Biodiesel Feedstock. Energies 33 (2008): 1646-1653.

Gunawan, F., et al. Synthesis of Biodiesel from Vegetable Oils Wastewater Sludge by in-Situ Subcritical Methanol Transesterification: Process Evaluation and Optimization. Biomass Bioenerg 69 (2014): 28-38.

Gunstone, F. D., Hamilton, R. J., Cast, J., Jee, M., and Hamm, W. Vegetable Oils in Food Technology: Composition, Properties and Uses. Florida: CRC Press, 2000.

Haagenson, D. M., Brudvik, R. L., Lin, H., and Wiesenborn, D. P. Implementing an in Situ Alkaline Transesterification Method for Canola Biodiesel Quality Screening. J Am Oil Chem Soc 87 (2010): 1351-1358.

Haagenson, D. M., and Wiesenborn, D. P. Impact of the North Dakota Growing Location on Canola Biodiesel Quality. J Am Oil Chem Soc 88 (2011): 1439-1445.

Haile, M., Asfaw, A., and Asfaw, N. Investigation of Waste Coffee Ground as a Potential Raw Material for Biodiesel Production. Int J Renew Energy Res 3,4 (2013): 854-860.

Hass, M. J., and Scott, K. M. Moisture Removal Substantially Improves the Efficiency of in Situ Biodiesel Production from Soybeans. J Am Oil Chem Soc 84 (2007): 197-204.

Hass, M. J., Scott, K. M., Marmer, W. N., and Foglia, T. A. In Situ Alkaline Transesterification: An Effective Method for the Production of Fatty Acid Esters from Vegetable Oils. J Am Oil Chem Soc 81,1 (2004): 83-89.

Havlik, P., et al. Global Land-Use Implication of Firsts and Second Generation Biofuel Target. Energy Policy 39 (2011): 5690-5702.

Hu, J., Du, Z., Tang, Z., and Min, E. Study on the Solvent Power of a New Green Solvent: Biodiesel. Journal of Industrial & Engineering Chemistry Research 43 (2004): 7928-7931.

Huang, G., Chen, F., Wei, D., Zhang, X., and Chen, G. Biodiesel Production by Microalgal Biotechnology. Applied Energy 87 (2009): 38-46.

International Coffee Organization. World Coffee Consumption. 2016. International Coffee Organization. Available: http://www.ico.org/prices/new-consumption-table.pdf. 19th May 2016.

International Grain Council. Grain Market Trends in the Stockfeed and Biodiesel Industries. 2008. Available: http://www.ausgrain.com.au/Back%20Issues/176magrn08/30_Market.pdf. 23 November 2012.

Isayama, Y., and Saka, S. Biodiesel Production by Supercritical Process with Crude Bio-Methanol Prepared by Wood Gasification. Bioresour Technol 99,11 (2008): 4775-4779.

ISO 14040. "Environmental Management." Life cycle assessment: Principles and framework. Geneva: International Organization for Standardization ISO, 2006.

ISO 14044. "Environmental Management." Life cycle assessment: Requirements and guidelines. Geneva: International Organization for Standardization ISO, 2006.

Kadioglu, S. I., Phan, T. T., and Sabatini, D. A. Surfactant-Based Oil Extraction of Corn Germ. Journal of American Oil Chemists' Society 88 (2011): 863-869.

Kaewcharoensombat, U., Prommetta, K., and Srinophakun, T. Life Cycle Assessment of Biodiesel Production from Jatropha. J Taiwan Inst Chem E 42 (2011): 454-462.

Kim, Y. H., Sohngen, B., Golub, A., Hertel, T. W., and Rose, S. "Impact of Us and European Biofuel Policies on Forest Carbon." Agricultural & Applied Economics Association's 2010 AAEA, CAES & WAEA Joint Annual Meeting. Denver, Colorado, USA, 2010.

Knothe, G., and Dunn, R. O. Biodiesel: An Alternative Diesel Fuel from Vegetable Oils or Animal Fats. In S. Z. Erhan (eds.), Industrial Uses of Vegetable Oils, pp.42-89. Champaign, Illinois: AOCS Press, 2005.

Knothe, G., Krahl, J., and Van Gerpen, J. The Biodiesel Handbook. Champaign: AOCS Press, 2005.

Kondamudi, N., Mohapatra, S. K., and Misra, M. Spent Coffee Grounds as a Versatile Source of Green Energy. J Agric Food Chem 56 (2008): 11757-11760.

Kwon, E. E., Yi, H., and Jeon, Y. J. Sequential Co-Production of Biodiesel and Bioethanol with Spent Coffee Grounds. Bioresour Technol 136 (2013): 475-480.

Landucci, G., et al. "Hazards Assessment of Vegetable Oil Storage and Processing Plants." ICheaP10. Florence, Italy, 2011.

Lankoski, J., and Ollikainen, M. "Biofuel Policies and the Environmental: The Effect of Biofuel Feedstock Production on Climate, Water Quality and Biodiversity." International Association of Agricultural Economists Conference. Beijing, China, 2009.

Madras, G., Kolluru, C., and Kumar, R. Synthesis of Biodiesel in Supercritical Fluids. Journal of Fuel 83 (2004): 2029-2033.

Mahajan, S., Konar, S. K., and Boocock, D. G. B. Variables Affecting the Production of Standard Biodiesel. Journal of American Oil Chemists Society 84 (2008): 189-195.

Marcus, Y. Principles of Solubility and Solutions. In J. Rydberg, C. Musikas and G. R. Choppin (eds.), Principles and Practices of Solvent Extraction, pp.21-70. New York, NY: Marcel Dekker, Inc., 1992.

Meziane, S., and Kadi, H. Kinetics and Thermodynamics of Oil Extraction from Olive Cake. J Am Oil Chem Soc 85 (2008): 391-396.

Miller, N. J., and Mudge, S. M. The Effect of Biodiesel on the Rate of Removal and Weathering Characteristics of Crude Oil within Artificial Sand Columns. Journal of Spill Science & Technology Bulletin 4,1 (1997): 17-33.

Mohammed-Dabo, I. A., Ahmad, M. S., Hamza, A., Muazu, K., and Aliyu, A. Cosolvent Transesterification of *Jatropha Curcas* Seed Oil. Journal of Petroleum Technology and Alternative Fuels 3,4 (2012): 42-51.

Monono, E. M., Webster, D. C., and Wiesenborn, D. P. Pilot Scale (10 Kg) Production and Characterization of Epoxidized Sucrouse Soyate. Ind Crop Prod 74 (2015): 987-997.

Naidu, M. M., Sulochanamma, G., Sampathu, S. R., and Srinivas, P. Studies on Extraction and Antioxidant Potential of Green Coffee. Food Chem. 107 (2008): 377-384.

Naksuk, A., Sabatini, D. A., and Tongcumpou, C. Microemulsion-Based Palm Kernel Oil Extraction Using Mixed Surfactant Solutions. Industrial Crops and Products 30 (2009): 194-198.

Nazir, N., Mangunwidjaja, D., Yarmo, M. A., and Malakul, P. Life Cycle Assessment Studies of Jatropha (*Jatropha Curcas*) Biodiesel Production Processed by in-Situ Transesterification Method. AIJSTPME 5,2 (2012): 13-20.

Nguyen, T., Do, L., and Sabatini, D. A. Biodiesel Production Via Peanut Oil Extraction Using Diesel-Based Reverse-Micellar Microemulsions. Fuel 89 (2010): 2285-2291.

OECD-FAO Agricultural Outlook. Biofuel Production 2010-19. 2010. Available: http://www.oecd.org/site/oecd-faoagriculturaloutlook/biofuelproduction2010-19.htm. 23 November 2012.

Ploysrimongkol, J., and Tongcumpou, C. Optimising Palm Oil-Ethanol Microemulsion for Use as Biofuel. J. Sci. Res. Chula. Unit. 34,1 (2009): 29-35.

Pradhan, A., et al. Energy Life-Cycle Assessment of Soybean Biodiesel Revisited. T ASABE 54,3 (2011): 1031-1039.

Pramanik, K. Properties and Use of Jatropha Curcas Oil and Diesel Fuel Blends in Compression Ignition Engine. Journal of renewable and Sustainable Energy 28 (2003): 239-248.

Preechajarn, S. Thailand Oilseeds and Products Annual. 2012. USDA Foreign Agricultural Service. Available: http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Oilseeds%20and%20Products%20Annual_Bangkok_Thailand_4-4-2012.pdf. 6 December 2012.

Qian, J., Wang, F., Liu, S., and Yun, Z. In Situ Alkaline Transesterification of Cattonseed Oil Production of Biodiesel and Nontoxic Cottonseed Meal. Bioresour Technol 99 (2008): 9009-9012.

Ramalakshmi, K., Rao, L. J. M., Ishikawa, Y. T., and Goto, M. Bioactivities of Low-Grade Green Coffee and Spent Coffee in Different in Vitro Model Systems. Food Chem 115 (2009): 79-85.

Rocha, M. V. P., et al. Ultrasound-Assisted Production of Biodiesel and Ethanol from Spent Coffee Grounds. Bioresour Technol 167,343-348 (2014).

Saunders, C., Blake, W. K., Marshall, L., Greenhaigh, S., and Pereira, M. A. Impact of a United States's Biofuel Policy on New Zealand's Agricultural Sector. Energy Policy 37 (2009): 3448-3454.

Schumacher, L. Biodiesel Lubricity. In G. Knothe, J. Krahl and J. Van Gerpen (eds.), The Biodiesel Handbook, pp.137-144. Champaign, IL: AOCS Press, 2005.

Silvia, S., Gassman, P. W., Jha, M., Kurkalova, L., and Kling, C. L. "Environmental and Rural Development Impact: The Water Quality Effect of Corn Ethanol Vs Switchgrass Based Biofuel in the Midwest." Collaboration conference of Farm Foundation, USDA Office and Energy Poilicy and New Uses, USDA Economic Research Service, USDA Rural Development, USDA Natural Resource Conservation Service, and U.S. Forest Service. St.Louis, Missouri, USA, 2008.

Singh, G., Pai, R. S., and Devi, V. K. Response Surface Methodology and Process Optimization of Sustained Release Pellets Using Taguchi Orthogonal Array Design and Central Composite Design. Journal of Advanced Pharmaceutical Techonology & Research 3,1 (2012): 30-40.

Singh, P. P., Maier, D. E., Okos, M. R., Cattanach, E., and Trumble, K. P. Effect of Physical Properties and Operating Parameters on Soybean Flaking. J Am Oil Chem Soc 76,8 (1999): 981-987.

Sonnemann, G., Castells, F., and Schuhmacher, M. Integrated Life-Cycle and Risk Assessment for Industrial Processes. Boca Raton, FL: Lewis Publishers, 2004.

Srivastava, A., and Prasad, R. Triglycerides-Based Diesel Fules. Journal of Renewable and Sustainable Energy Reviews 4 (2000): 111-133.

Sua´rez-Quiroz, M. L., et al. Isolation of Green Coffee Chlorogenic Acids Using Activated Carbon. J. Food Compos. Anal. 33 (2014): 55-58.

The Trade News Service. Daily Price Record for Fats and Oils of 5 Oct 2012. 2012. Available: http://www.fats-and-oils.com/samples/tnsdaily.pdf. 27 November 2012.

Tuntiwiwattanapun, N., Tongcumpou, C., and Wiesenborn, D. Optimization of Alcoholic Soybean Oil Extraction as a Step Towards Developing in-Situ Transesterification for Fatty Acid Isopropyl Ester. Ind Crop Prod 94 (2016): 189-196.

Twarath, S. "Updated Renewable Energy Policies : Case of Thailand." 2013 International Conference on Alternative Energy in Development Countries and Emerging Economies (2013 AEDCEE). Bangkok, Thailand: Department of Alternative Energy Development and Efficiency, 2013.

USDA. World: Soybeans and Products Supply and Distribution 2012. Available: http://www.fas.usda.gov/psdonline/psdreport.aspx?hidReportRetrievalName=BVS&hidReportRetrievalID=715&hidReportRetrievalTemplateID=13. 6 December 2012.

Van Gerpen, J., and Knothe, G. Basic of the Transesterification Reaction. In G. Knothe, J. Krahl and J. Van Gerpen (eds.), The Biodiesel Handbook, pp.26-41. Champaign: AOCS Press, 2005.

Varanda, M. G., Pinto, G., and Martin, F. Life Cycle Analysis of Biodiesel Production. Fuel Process Technol 92 (2011): 1087-1094.

Vardon, D. R., et al. Complete Utilization of Spent Coffee Grounds to Produce Biodiesel, Bio-Oil, and Biochar. ACS Sustainable Chem. Eng. 1 (2013): 1286-1294.

Walker, K. Non-Food Uses. In F. D. Gunstone (eds.), Rapeseed and Canola Oil: Production, Processing, Properties and Uses, Florida: CRC Press, 2004.

Wan Omar, W. N. N., Nordin, N., Mohamed, M., and Amin, N. A. S. A Two-Step Biodiesel Production from Waste Cooking Oil: Optimization of Pre-Treatment Step. Journal of Applied Sciences 9,17 (2009): 3098-3103.

Wang, P. S., Tat, M. E., and Van Gerpen, J. The Production of Fatty Acid Isopropyl Esters and Their Use as a Diesel Engine Fuel. J Am Oil Chem Soc 82,11 (2005): 845-849.

Wides, S. Methyl Soyate: A New Green Alternative Solvent. Division of Chemical Health and Safety of the American Chemical Society 5/6 (2002): 25-26.

Wisner, R. Biodiesel Economic: Costs, Tax Credits and Co-Product. June 2009 2009. Agricultural marketing resource center. Available: http://www.agmrc.org/renewable_energy/biodiesel/biodiesel-economics-costs-tax-credits-and-co-product/. 24 SEP 2014.

Worapun, I., Pianthong, K., and Thaiyasuit, P. Synthesis of Biodiesel by Two-Step Transesterification from Crude *Jatropha Curcus* L. Using Ultrasonic Irradiation Assisted. Journal of KKU Engineering 37,3 (2010): 169-179.

Yusuf, N. N. A. N., Kamarudin, S. K., and Yaakub, Z. Overview on the Current Trends in Biodiesel Production. Energy Conversion and Management 52 (2011): 2741-2751.

Zakaria, R., and Harvey, A. P. Kinetics of Reactive Extraction/in Situ Transesterification of Rapeseed Oil. Fuel Process Technol 125 (2014): 34-40.

APPENDIX

APPENDIX A

ANOVA TABLE

Table A1 ANOVA results of triglyceride extraction from screening the most crucial factors of *in-situ* TE in part 1.

Effect	Analysis of Variance (Taguchi L18 for *in-situ* TE) Mean = 38.1953 Sigma = 3.12531				
	SS	df	MS	F	p
Catalyst Type	20.9737	1	20.97372	29.6847	0.000003
Temperature (°C)	24.5258	2	12.26292	17.3561	0.000004
Time (min)	59.6523	2	29.82616	42.2138	0
Shaking speed (rpm)	21.6035	2	10.80175	15.288	0.000013
Catalyst Concentration(% w/v)	84.2332	2	42.11658	59.6087	0
Moisture in oilseed (% w/w)	13.0364	2	6.51821	9.2254	0.000542
S-L ratio (g/mL)	143.2459	2	71.62297	101.3699	0
Acetone Proportion (% v/v)	123.5607	2	61.78035	87.4394	0
Residual	26.8489	38	0.70655		

Table A2 ANOVA results of biodiesel yield from screening the most crucial factors of *in-situ* TE in part 1.

Effect	Analysis of Variance (Taguchi L18 for *in-situ* TE) Mean = 35.5854 Sigma = 5.65060				
	SS	df	MS	F	p
Catalyst Type	124.34	1	124.34	20.24562	0.000063
Temp (°C)	33.4472	2	16.7236	2.72302	0.078494
Time (min)	215.6413	2	107.8206	17.55587	0.000004
Shaking speed (rpm)	31.3534	2	15.6767	2.55255	0.091168
Catalyst Concentration (% w/v)	513.47	2	256.735	41.80281	0
Moisture in seed (% w/w)	29.5664	2	14.7832	2.40707	0.103689
S-L ratio (g/mL)	358.1751	2	179.0876	29.15989	0
Acetone Proportion (% v/v)	152.8787	2	76.4394	12.44622	0.00007
Residual	233.3797	38	6.1416		

Table A3 ANOVA results of biodiesel yield from optimizing soybean biodiesel production via *in-situ* TE using methanol with acetone as co-solvent in part 2.

Factor	ANOVAR: Biodiesel Yield (%); R-sqr=.93981; Adj:.80739 (CCRD for Methanol-Acetone *in-situ* TE) 3 factors, 3 Blocks, 17 Runs; MS Residual=11.36285				
	SS	df	MS	F	p
Blocks	134.3475	2	67.1737	5.91170	0.048155
Time (min) (X_1)	27.3771	1	27.3771	2.40935	0.181316
Time (min) (X_1^2)	21.5177	1	21.5177	1.89369	0.227225
Liquid-Solid Ratio (mL/g) (X_2)	505.7721	1	505.7721	44.51102	0.001143
Liquid-Solid Ratio (mL/g) (X_2^2)	53.0315	1	53.0315	4.66709	0.083151
Acetone (% v/v) (X_3)	71.0570	1	71.0570	6.25345	0.054444
Acetone (% v/v) (X_3^2)	0.5320	1	0.5320	0.04682	0.837251
X_1 by X_2	8.6830	1	8.6830	0.76416	0.422024
X_1 by X_3	2.4860	1	2.4860	0.21879	0.659645
X_2 by X_3	63.9672	1	63.9672	5.62950	0.063742
Error	56.8143	5	11.3629		
Total SS	943.8833	16			

Table A4 ANOVA results of biodiesel yield from optimizing soybean biodiesel production via *in-situ* TE using methanol in part 2.

Factor	ANOVA: Biodiesel yield; R-sqr=.89601; Adj:.76601 (CCRD for Methanol for *in-situ* TE) 2 factors, 1 Blocks, 10 Runs; MS Pure Error=2.361752				
	SS	df	MS	F	p
L-S ratio (mL/g) (X_1)	283.6	1	283.6	120.0805	0.057935
L-S ratio (mL/g) (X_1^2)	247.478	1	247.478	104.7858	0.061994
Na (metal) Concentration (% w/v) (X_2)	148.977	1	148.977	63.079	0.079737
Na (metal) Concentration (% w/v) (X_2^2)	1080.571	1	1080.571	457.5295	0.029741
X_1 by X_2	94.138	1	94.138	39.8593	0.100005
Lack of Fit	184.57	3	61.523	26.0499	0.142813

Table A5 ANOVA results of biodiesel yield from optimizing soybean biodiesel production via *in-situ* TE using ethanol in part 2.

Factor	ANOVA: Biodiesel yield; R-sqr=.98751; Adj:.9719 (CCRD for Ethanol for *in-situ* TE) 2 factors, 1 Blocks, 10 Runs; MS Pure Error=.0002729				
	SS	df	MS	F	p
L-S ratio (mL/g) (X_1)	625.93	1	625.93	256.7784	0.000529
L-S ratio (mL/g) (X_1^2)	163.824	1	163.824	67.2062	0.003798
Na (metal) Concentration (% w/v) (X_2)	1176.236	1	1176.236	482.5328	0.000207
Na (metal) Concentration (% w/v) (X_2^2)	21.228	1	21.228	8.7085	0.059976
X_1 by X_2	0.662	1	0.662	0.2716	0.638257
Lack of Fit	24.287	3	8.096	3.3212	0.175275

Table A6 ANOVA results of biodiesel yield from optimizing soybean biodiesel production via *in-situ* TE using isopropanol in part 2.

Factor	ANOVA: Biodiesel yield; R-sqr=.97076; Adj:.93421 (CCRD for Isopropanol for in_situ TE) 2 factors, 1 Blocks, 10 Runs; MS Pure Error=2.544111				
	SS	df	MS	F	p
L-S ratio (mL/g) (X_1)	465.115	1	465.1147	182.8201	0.046998
L-S ratio (mL/g) (X_1^2)	109.896	1	109.8956	43.1961	0.096126
Na (metal) Concentration (% w/v) (X_2)	490.187	1	490.1869	192.6751	0.045784
Na (metal) Concentration (% w/v) (X_2^2)	40.633	1	40.6328	15.9713	0.156093
X_1 by X_2	241.939	1	241.9387	95.0975	0.065055
Lack of Fit	36.96	3	12.3198	4.8425	0.319614

Table A7 ANOVA results of biodiesel yield from optimizing soybean biodiesel production via *in-situ* TE using aqueous ethanol in part 2.

Factor	ANOVA: Biodiesel Yield (%); R-sqr=.97445; Adj:.9425 (CCRD_Aqueous Ethanol vs Temperature of *in-situ* TE) 2 factors, 1 Blocks, 10 Runs; MS Pure Error=.8806252				
	SS	df	MS	F	p
Water Content in Ethanol (%w/v) (X_1)	2358.362	1	2358.362	2678.055	0.0123
Water Content in Ethanol (%w/v) (X_1^2)	877.092	1	877.092	995.988	0.020165
Temperature (°C) (X_2)	127.325	1	127.325	144.585	0.052823
Temperature (°C) (X_2^2)	6.168	1	6.168	7.004	0.229999
X_1 by X_2	161.41	1	161.41	183.291	0.046938
Lack of Fit	94.887	3	31.629	35.917	0.121907

Table A8 ANOVA results of triglyceride in biodiesel from optimizing soybean biodiesel production via *in-situ* TE using aqueous ethanol in part 2.

Factor	ANOVA: Triglyceride in Biodiesel (% wt); R-sqr=.9886; Adj:.97435 (CCRD_Aqueous Ethanol vs Temperature of *in-situ* TE) 2 factors, 1 Blocks, 10 Runs; MS Pure Error=.1051634				
	SS	df	MS	F	p
Water Content in Ethanol (%w/v) (X_1)	215.0039	1	215.0039	2044.475	0.014077
Water Content in Ethanol (%w/v) (X_1^2)	60.7243	1	60.7243	577.428	0.026478
Temperature (°C) (X_2)	20.2576	1	20.2576	192.629	0.045790
Temperature (°C) (X_2^2)	1.6610	1	1.6610	15.795	0.156928
X_1 by X_2	19.3142	1	19.3142	183.659	0.046891
Lack of Fit	3.5908	3	1.1969	11.382	0.213750

Table A9 ANOVA results of oil content in DSCG after deacidification process in part 3.

Factor	ANOVA: Oil in DSCG (% wt); R-sqr=.88728; Adj:.74639 (CCRD for Deacidification (MeOH)) 2 factors, 1 Blocks, 10 Runs; MS Pure Error=1.611888				
	SS	df	MS	F	p
L-S ratio (mL/g) (X_1)	31.66995	1	31.66995	19.64774	0.141258
L-S ratio (mL/g) (X_1^2)	8.19116	1	8.19116	5.08172	0.265803
Temperature (°C) (X_2)	0.28104	1	0.28104	0.17435	0.748186
Temperature (°C) (X_2^2)	3.66536	1	3.66536	2.27395	0.37278
X_1 by X_2	1.81561	1	1.81561	1.12639	0.481069
Lack of Fit	3.7919	3	1.26397	0.78415	0.659078

Table A10 ANOVA results of acid value in DSCG oil after deacidification process in part 3.

Factor	ANOVA: Acid Value; R-sqr=.92583; Adj:.83311 (CCRD for Deacidification (MeOH)) 2 factors, 1 Blocks, 10 Runs; MS Residual=.021366				
	SS	df	MS	F	p
L-S ratio (mL/g) (X_1)	0.905493	1	0.905493	42.38013	0.002874
L-S ratio (mL/g) (X_1^2)	0.098892	1	0.098892	4.62848	0.097829
Temperature (°C) (X_2)	0.029895	1	0.029895	1.39919	0.302363
Temperature (°C) (X_2^2)	0.00041	1	0.00041	0.0192	0.896496
X_1 by X_2	0.00306	1	0.00306	0.14324	0.724315

Table A11 ANOVA results of biodiesel yield from optimizing SCG biodiesel production via *in-situ* TE in part 3.

Effect	ANOVA: Biodiesel Yield (%) Full factor design (SCG Biodiesel): 2 factors, 4 levels				
	SS	df	MS	F	p
DSCG Particle Size (mm)	4162.6	3	1387.5	254.04	0
Temperature (°C)	2455.2	3	818.4	149.84	0
Interaction	869.6	9	96.6	17.69	0.000001
Error	87.4	16	5.5		

APPENDIX B

HPLC-ELSD CONDITIONS AND THE CHROMATOGRAM OF EACH COMPOUND

A Shimadzu-HPLC with auto injector (model Shimadzu-10Avp, Japan) and a Sedere-ELSD (model Sedex 75, France) were operated under the following conditions: a C18 column, 5 µm, 4.6×250 mm (Inertsil® ODS-3, Japan) was warmed to 70 °C; the mobile phase was a mixture of methanol and isopropanol (gradient elution: starting at 100% methanol and ending at 15% methanol after 30 min) with a flow rate of 0.75 cm^3 min^{-1}; the detection temperature and pressure of the ELSD were 40 °C and 210-220 kPa, respectively; and the injection volume was 0.2 mm^3.

Despite of triglyceride and biodiesel, this HPLC-ELSD conditions could be used to identify several compounds related to TE reaction, for example FFA, glycerin, mono-glycerol and di-glycerol. The chromatograms of each compound were illustrated below.

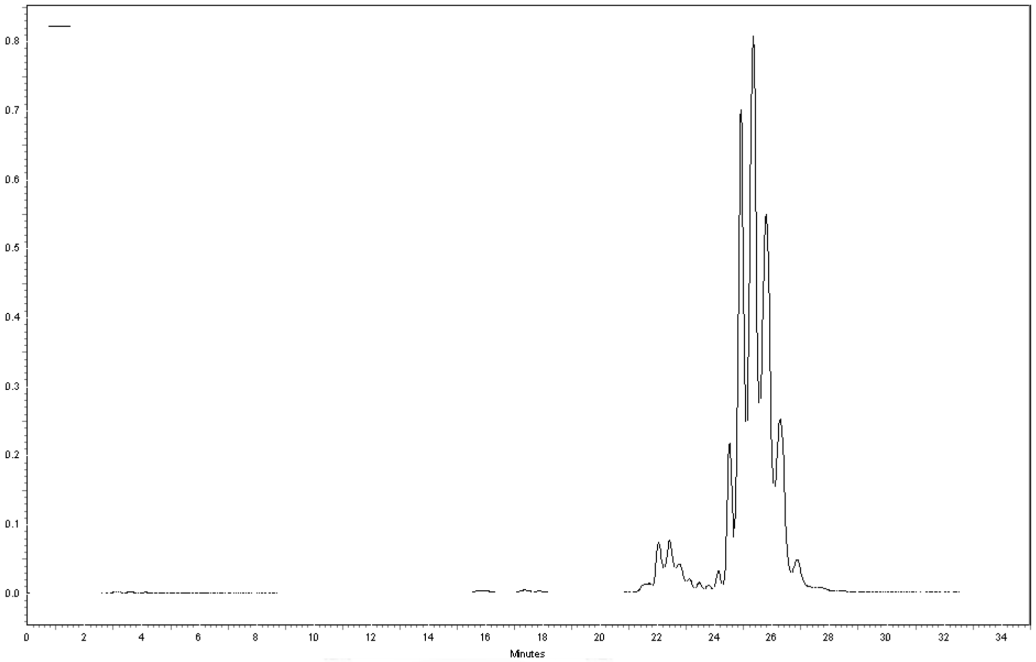


Figure B1 Chromatogram of refined soybean oil (triglyceride).

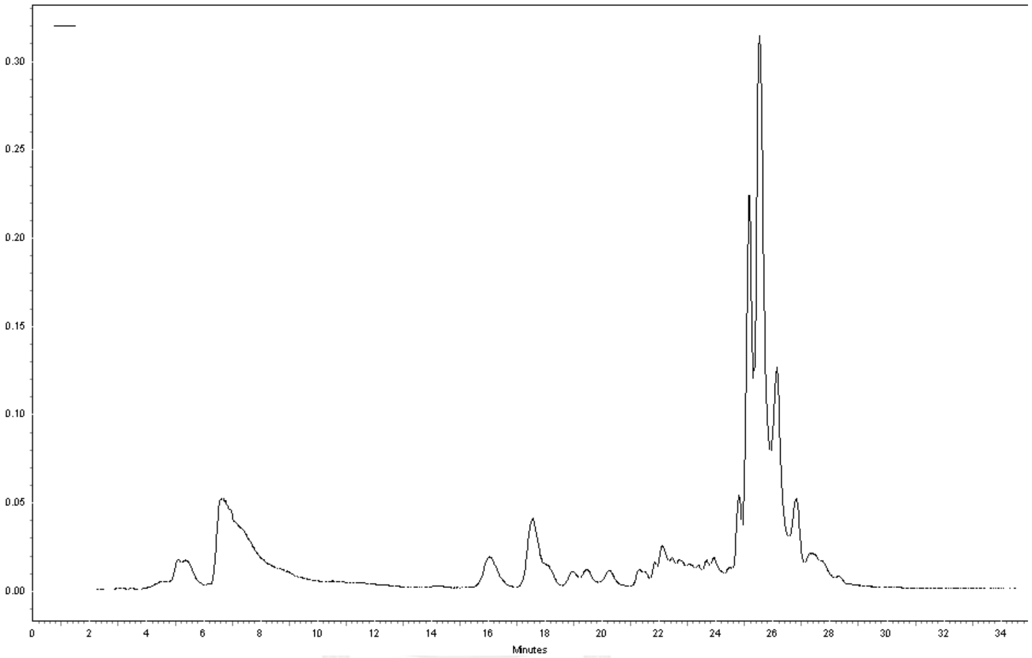


Figure B2 Chromatogram of crude SCG oil by Soxhlet n-hexane extraction.

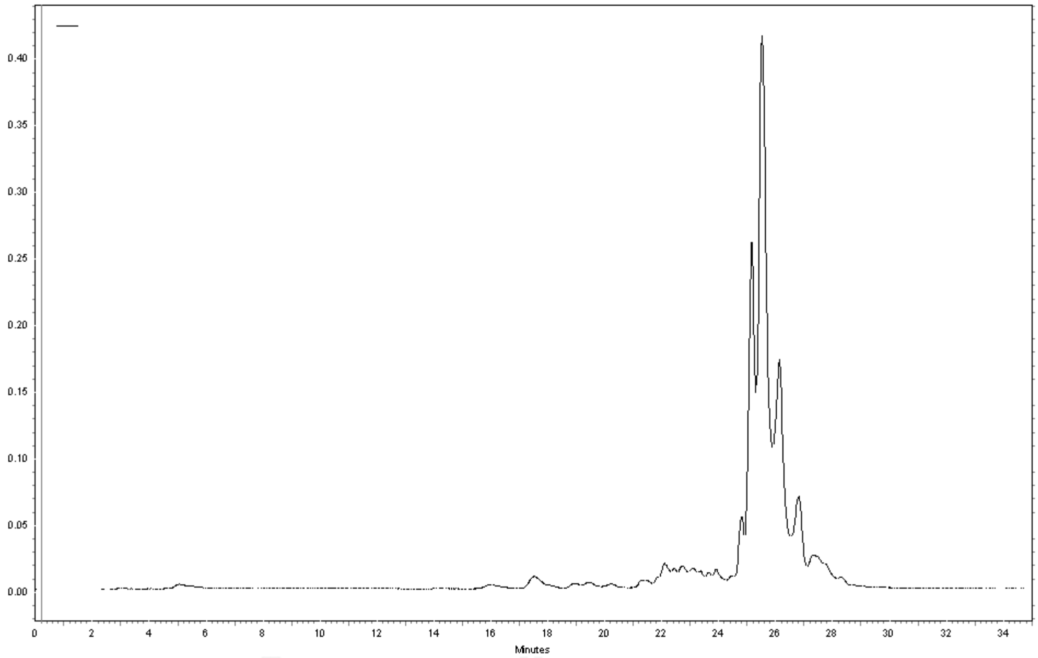


Figure B3 Chromatogram of DSCG oil by methanol washing and Soxhlet n-hexane extraction.

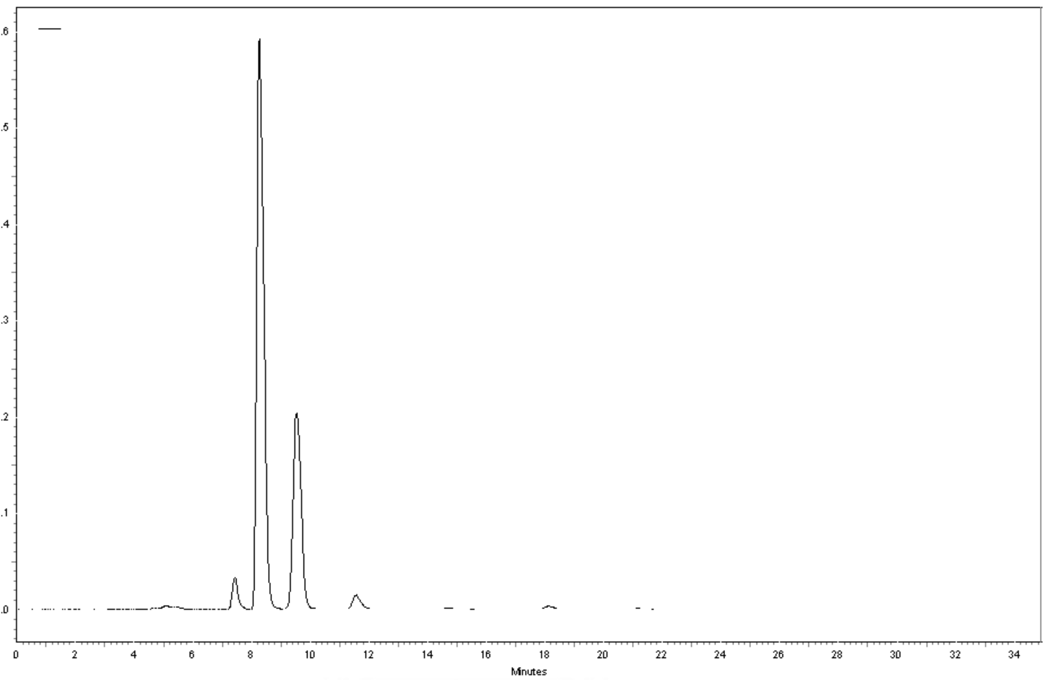


Figure B4 Chromatogram of fatty acid methyl ester produced from refined soybean oil.

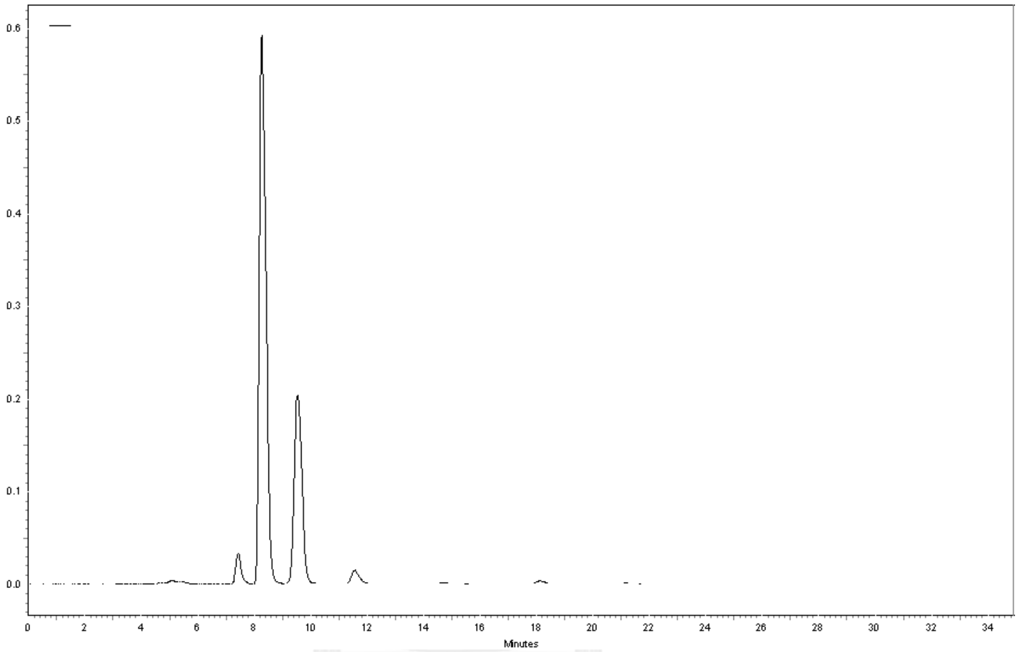


Figure B5 Chromatogram of fatty acid methyl ester produced from ground soybean via *in-situ* TE.

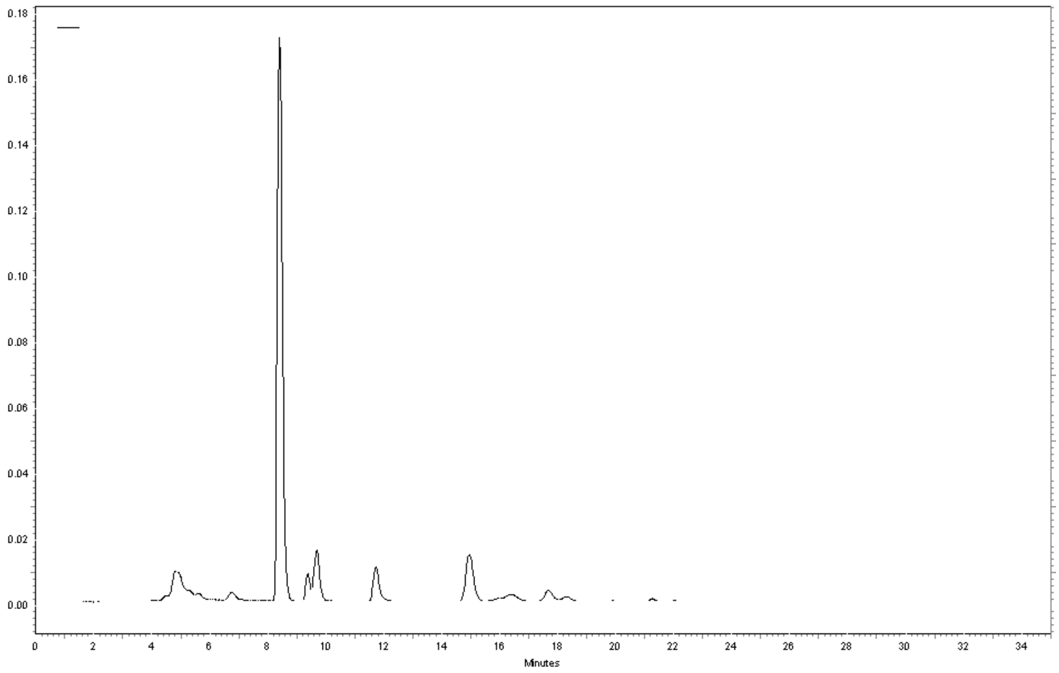


Figure B6 Chromatogram of fatty acid methyl ester produced from SCG via *in-situ* TE.

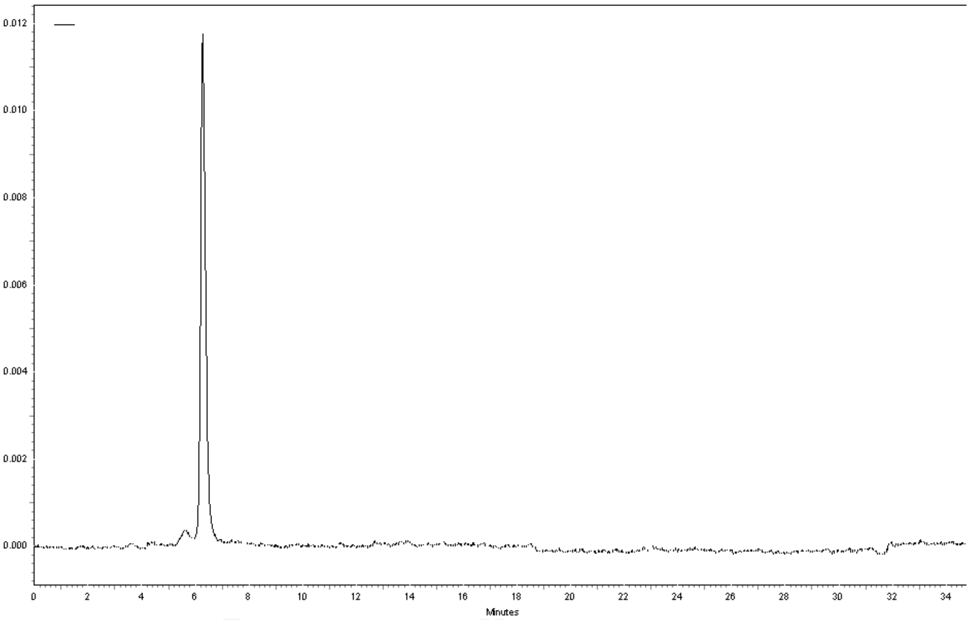


Figure B7 Chromatogram of mono-olein standard

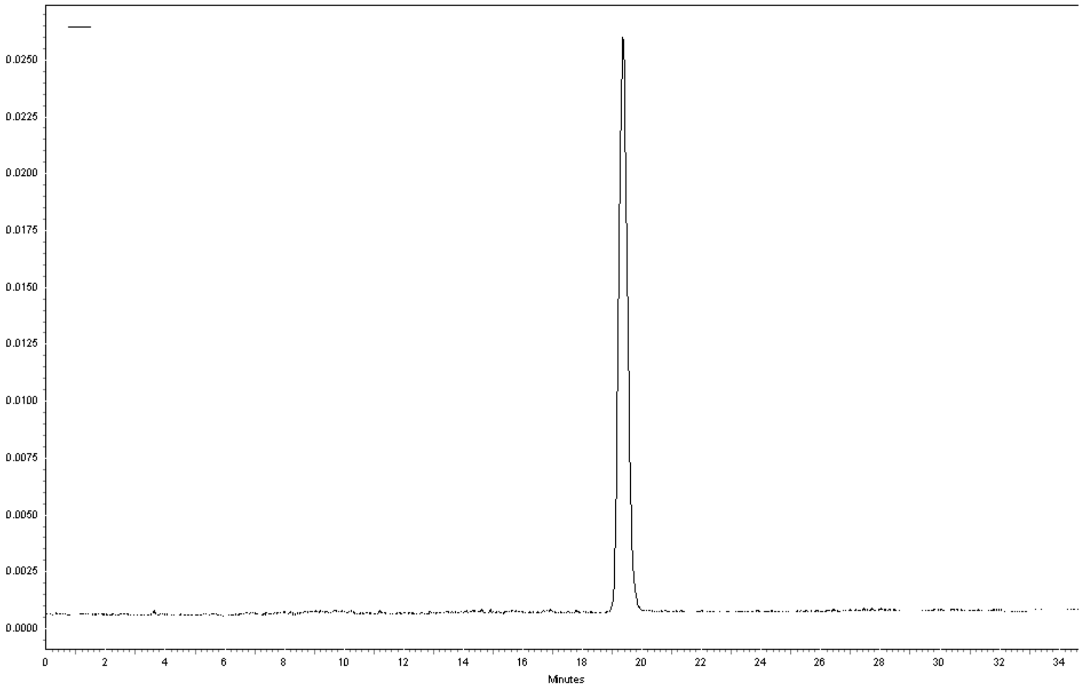


Figure B8 Chromatogram of di-olein standard.

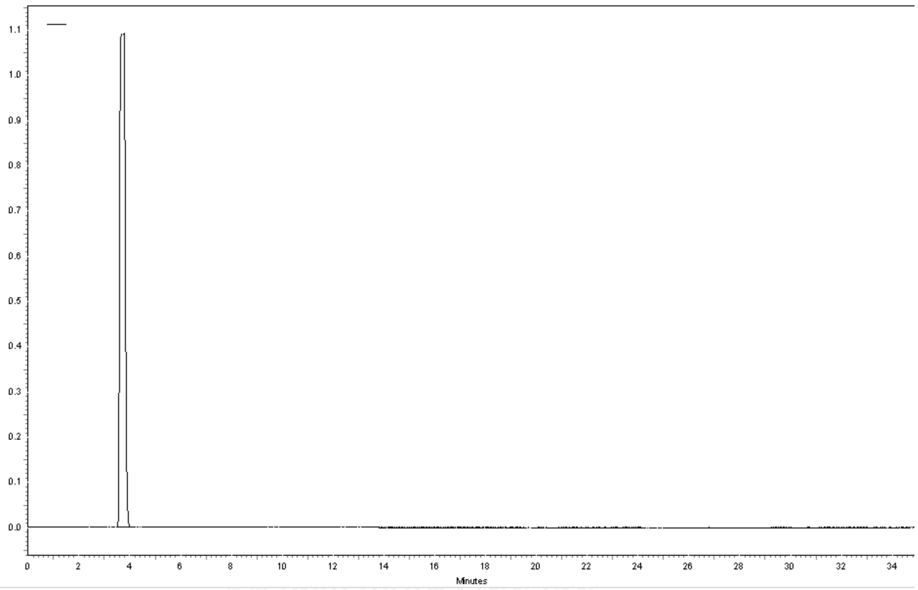


Figure B9 Chromatogram of glycerin (industrial grade).

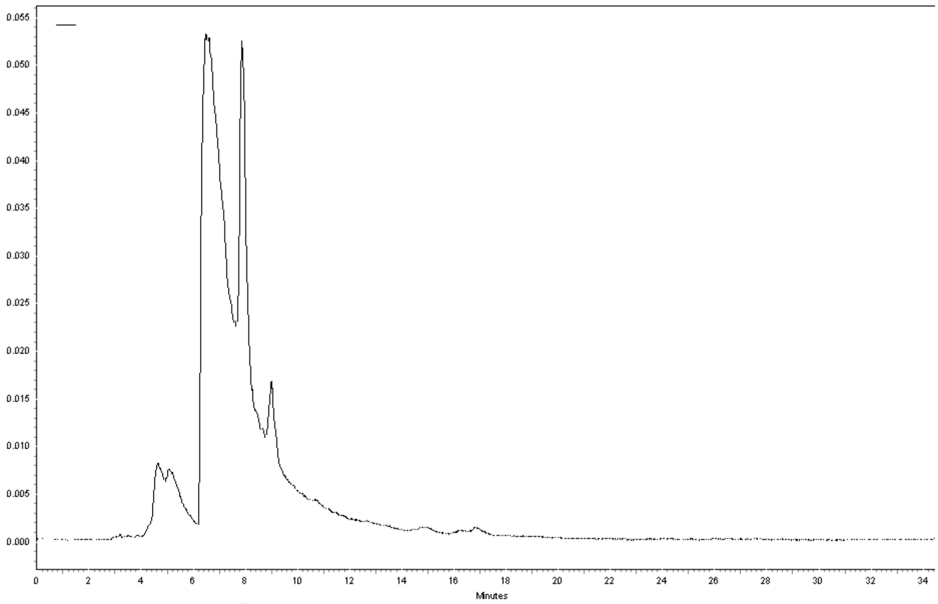


Figure B10 Chromatogram of FFA of soybean oil produced by saponification and neutralization with acid.

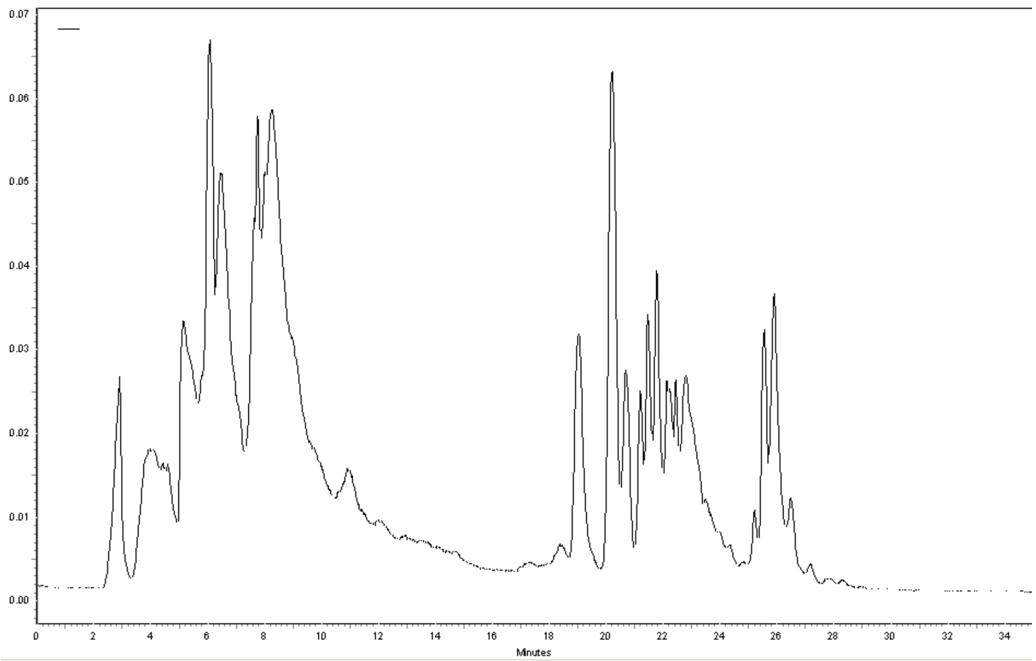


Figure B10 Chromatogram of liquid fraction from deacidification process of SCG by methanol washing.

APPENDIX C

REACTOR AND MIXER USED IN THIS STUDY AND FUTURE WORK

Figure C1 Small scale mixer and water bath used in SCG biodiesel production.

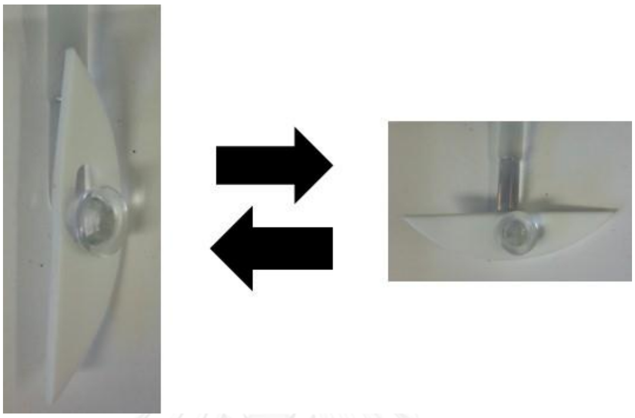


Figure C2 Adjustable 2-blade propeller for small scale mixer used in SCG biodiesel production.

Figure C3 Pilot scale 38 L reactor used in SCG biodiesel production for processing 4 kg SCG per batch.

Figure C4 Anchor type propeller used in pilot scale SCG biodiesel production.

Figure C5 Separation of defatted SCG from liquid fraction after *in-situ* TE by paper filtration.

Figure C6 Methanol evaporation of liquid fraction after in-situ TE using 12 L Soxhlet apparatus.

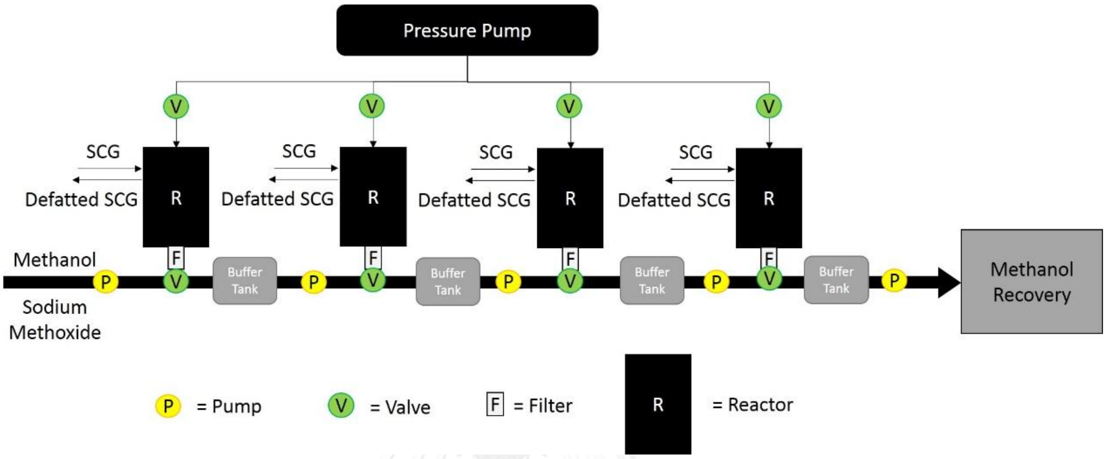


Figure C7 Concept diagram of semi-countercurrent extraction reactor for improving energy usage and environmental impacts of *in-situ* TE.

VITA

Mr. Nattapong Tuntiwiwattanapun was born on March 15, 1985 in Bangkok, Thailand. He graduated his Bachelor's degree in Food Technology from faculty of Science, Chulalongkorn University in 2006. In 2012, he achieved two Master's degrees in Science from (1) International Programs in Hazardous Substance and Environmental Management, Graduate School, Chulalongkorn University; and (2) Environmental and Conservation Sciences Program, North Dakota State University. His work was published in the Journal of American Chemistry's Society in the title of "Develop and Scale-up Aqueous Surfactant-assisted Extraction of Canola Oil for Used as Biodiesel Feedstock" in 2013.

After graduated in Master's degree, he pursued his Ph.D. in 2012 and received the Golden Royal Jubilee Scholarship. He gained an opportunity to participate in the workshop "2013 ProSper.Net Young Researchers' School" organized by United Nation University in the theme of "Partnerships in Water and Biodiversity for Sustainable Development." In 2014, he went to Japan for a short course study in "Renewable Energy" organized by United Nation University. In 2015, he went to North Dakota State University to collaborate his work for 6 months. During his Ph.D., he gave 2 posters and 2 oral presentations; and he obtained the outstanding in oral presentation at RGJ-Ph.D. Congress 17th, Thailand, June 8-11, 2016. For the publication, his first paper was published by Industrial Crops and Products in the title of "Optimizing of Alcoholic Soybean Oil Extraction as a Step towards Developing In-situ Transesterification for Fatty Acid Isopropyl Ester" in 2016. The second manuscript was submitted to Fuel in the title of "Developing an In-situ Transesterification Process for Biodiesel Production Using Spent Coffee Grounds form the Instant Coffee Industry" in July, 2016. He got the privilege to receive the pin of honor from Chulalongkorn University as the outstanding graduate student in 2016.