

DEVELOPMENT AND SCALE UP OF AQUEOUS SURFACTANT-ASSISTED  
EXTRACTION OF CANOLA OIL FOR USE AS BIODIESEL FEEDSTOCK

A Thesis  
Submitted to the Graduate Faculty  
of the  
North Dakota State University  
of Agriculture and Applied Science

By

Nattapong Tuntiwiwattanapun

In Partial Fulfillment  
for the Degree of  
MASTER OF SCIENCE

Major Program:  
Environmental and Conservation Sciences

August 2012

Fargo, North Dakota

North Dakota State University  
Graduate School

---

**Title**

Development and Scale Up of Aqueous Surfactant-assisted Extraction of

---

Canola Oil for Use as Biodiesel Feedstock

---

**By**

Nattapong Tuntiwiwattanapun

---

The Supervisory Committee certifies that this *disquisition* complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

---

SUPERVISORY COMMITTEE:

Dr. Dennis Wiesenborn

---

Chair

Dr. Eakalak Khan

---

Dr. Scott Pryor

---

Dr. Zhulu Lin

---

Approved:

15 NOV 2012

---

Date

Dr. Craig Stockwell

---

Department Chair

## **ABSTRACT**

In oilseed extraction, alternative extraction media have been studied to replace the traditional solvent, hexane, due to health, safety, and environmental concerns. In this work, aqueous surfactant-assisted extraction was developed and scaled up for hexane-free canola oil extraction. An inexpensive commercial surfactant system was formulated and used as an extraction medium based on the dynamic interfacial tension value. The extraction conditions were evaluated to achieve the maximum oil recovery. The highest oil detachment efficiency was 80% in the absence of hexane at room temperature. The extracted oil had acceptable content of free fatty acids, water and phospholipids for use as a biodiesel feedstock. The biodiesel product passed the ASTM D6751 biodiesel standard for water content, kinematic viscosity, acid value and oxidative stability.

## ACKNOWLEDGMENTS

I would like to gratefully thank my advisor Dr. Dennis P. Wiesenborn, Professor in the Department of Agricultural and Biosystems Engineering, for his support and guidance through the duration of my graduate study in NDSU. To be an advisee of Dr. Dennis was the most precious experience in my life. I also would like to express my appreciation to Dr. Chantra Tongcumpou, my co-advisor in the Environmental and Hazardous Waste Management Program of Chulalongkorn University (Thailand), for her support and encouragement. It was a great honor to work with them.

I would like to extend my acknowledgement and appreciation to my advisory committees including Dr. Eakalak Khan, Professor in the Department of Civil Engineering; Dr. Scott W. Pryor, Associate Professor in the Department of Agricultural and Biosystems Engineering; and Dr. Zhulu Lin, Assistant Professor in the Department of Agricultural and Biosystems Engineering. Without their advice and comments, this work would not be possible. Furthermore, special thanks go to Darrin M. Haagenon, a research specialist in the Department of Agricultural and Biosystems Engineering, who advised me on the biodiesel process, analytical methods and statistical analysis. I am grateful to Dr. Mukhlesur Rahman, canola breeder in the Department of Plant Science, for providing the canola seed. I extend my appreciation to the North Dakota Center of Excellence for Oilseed Development for providing funding.

Thanks to my family, friends and staff at NDSU and in Thailand, especially my parents, brother, sister and Manapa Akramas for their endless support and belief in me unconditionally.

## TABLE OF CONTENTS

ABSTRACT.....	iii
ACKNOWLEDGEMENTS.....	iv
LIST OF TABLES.....	xi
LIST OF FIGURES.....	xii
LIST OF ABBREVIATIONS.....	xv
LIST OF APPENDIX TABLES.....	xvi
CHAPTER 1. GENERAL INTRODUCTION.....	1
1.1. Introduction.....	1
1.2. Objective of the Study.....	5
1.3. Thesis Organization.....	5
1.4. References.....	6
CHAPTER 2. LITERATURE REVIEW.....	11
2.1. Technology and Solvent for Oilseed Extraction.....	11
2.1.1. Oilseed extraction process.....	12
2.1.1.1. Pretreatment steps.....	14
2.1.1.2. Mechanical extraction.....	14

2.1.1.3. Solvent extraction .....	16
2.2. Aqueous Surfactant-Assisted Extraction (ASE) .....	17
2.2.1. How to reduce interfacial tension .....	18
2.2.1.1. Critical micelle concentration .....	20
2.2.1.2. Winsor R ratio.....	20
2.2.2. Extraction process.....	26
2.3. Canola. ....	31
2.4. Biodiesel: From Farm to Fuel Concept.....	32
2.4.1. Foundation of transesterification reaction .....	33
2.4.2. Quality of feed stock for biodiesel production .....	35
2.4.3. Biodiesel quality and standard .....	35
2.4.4. Optimizing biodiesel production and quality.....	36
2.5. References.....	37
<b>CHAPTER 3. PAPER 1: IMPROVING CANOLA OIL DETACHMENT EFFICIENCY BY REDUCING INTERFACIAL TENSION .....</b>	<b>43</b>
3.1. Abstract.....	44
3.2. Introduction.....	44
3.3. Materials and Methods.....	47

3.3.1. Materials .....	47
3.3.2. Dynamic interfacial tension measurement.....	48
3.3.3. Hydrophile-lipophile balance value calculation .....	48
3.3.4. Oilseed pretreatment .....	49
3.3.5. Aqueous surfactant-assisted extraction (ASE).....	49
3.3.6. Oil content of oilseed and extracted meal.....	50
3.3.7. Experimental design.....	50
3.3.7.1. Anionic surfactant selection.....	50
3.3.7.2. Effect of additional nonionic surfactant.....	51
3.3.7.3. Effect of electrolyte.....	51
3.3.7.4. Effect of interfacial tension on ASE .....	51
3.3.8. Statistical analysis.....	51
3.4. Results and Discussion .....	52
3.4.1. Anionic surfactant selection .....	52
3.4.2. Effect of additional nonionic surfactant .....	53
3.4.3. Effect of electrolyte .....	55
3.4.4. Effect of interfacial tension on ASE .....	57

3.5.	Conclusions.....	59
3.6.	Acknowledgements.....	59
3.7.	References.....	60
CHAPTER 4. PAPER 2: DEVELOPMENT AND SCALE UP OF AQUEOUS SURFACTANT-ASSISTED EXTRACTION OF CANOLA OIL FOR USE AS BIODIESEL FEEDSTOCK .....		64
4.1.	Abstract.....	65
4.2.	Introduction.....	65
4.3.	Materials and Methods.....	69
4.3.1.	Materials .....	69
4.3.2.	Oilseed pretreatment .....	69
4.3.3.	Aqueous surfactant-assisted extraction (ASE).....	70
4.3.4.	Oil content of oilseed and extracted meal.....	71
4.3.5.	Experimental design.....	71
4.3.5.1.	Effect of surfactant concentration.....	71
4.3.5.2.	Effect of stirring time and ultrasonication .....	72
4.3.5.3.	Effect of extraction media and temperature.....	72
4.3.5.4.	Effect of solid-liquid ratio.....	73

4.3.5.5. Effect of triple extraction with three stages .....	73
4.3.6. Scale up of the ASE process .....	74
4.3.7. Other extraction methods .....	75
4.3.8. Transesterification.....	75
4.3.9. Extracted oil and biodiesel quality analysis .....	75
4.3.10. Statistical analysis .....	76
4.4. Results and Discussion .....	76
4.4.1. Effect of surfactant concentration .....	76
4.4.2. Effect of stirring time and ultrasonication .....	78
4.4.3. Effect of extraction media and temperature .....	79
4.4.4. Effect of solid-liquid ratio .....	81
4.4.5. Effect of triple extraction with three stages .....	81
4.4.6. Scale up of the ASE process .....	84
4.4.7. Extracted oil quality .....	86
4.4.7.1. Water content .....	86
4.4.7.2. Acid value .....	86
4.4.7.3. Phosphorus content .....	86

4.4.8. Biodiesel quality .....	87
4.4.8.1. Water content .....	87
4.4.8.2. Kinematic viscosity.....	87
4.4.8.3. Cloud point.....	87
4.4.8.4. Acid value .....	88
4.4.8.5. Oxidative stability index (OSI).....	88
4.5. Conclusions.....	90
4.6. Acknowledgements.....	91
4.7. References.....	91
CHAPTER 5. GENERAL CONCLUSIONS AND FUTURE WORK .....	97
5.1. General Conclusions .....	97
5.2. Future Work .....	98
APPENDIX A. EXPERIMENTAL DATA OF INTERFACIAL TENSION VALUE .....	100
APPENDIX B. ASE EXTRACTION CONDITIONS AND EXPERIMENTAL DATA.....	108
APPENDIX C. EXPERIMENTAL DATA OF EXTRACTED CANOLA OIL AND BIODIESEL QUALITY .....	116

## LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Numerical evaluation of attributes of solvents for vegetable oil extraction (Gregory and Horsman, 1997) .....	12
2. Oil content of various oilseeds with the prevalent method of extraction (Johnson, 1997).....	13
3. Summary of the extraction process and oil extraction efficiency of aqueous surfactant-assisted extraction method. ....	29
4. Summary of quality analysis of extracted vegetable oil from Table 3. ....	30
5. Biodiesel standard ASTM D6751 (American Society for Testing and Materials, 2009).....	36
6. HLB value and the dynamic IFT at optimum salinity of each surfactant system.....	57
7. Comparison of extraction conditions and oil efficiency between references and this work.....	85
8. Extracted oil and biodiesel product quality.. ....	90

## LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Conventional commercial vegetable oil extraction process (Booth, 2004) .....	13
2. Anatomy of a plant cell: vegetable oil is contained in vacuole (Davidson, 2011).....	15
3. Crown Model III oilseed extractor: <a href="http://www.crowniron.com/technologies/ext_model3.cfm">http://www.crowniron.com/technologies/ext_model3.cfm</a> . ....	16
4. Surfactant structure (Carlota et al., 2005).....	18
5. Molecule interaction energy at the interface between two pure liquid phases “a” and “b” (Rosen, 2004) .....	19
6. Effect of surfactant concentration on surface tension or interfacial tension (Kunjappu, 2003) .....	20
7. Effect of molecular environmental conditions on interfacial tension and phase volumes. Shaded phases indicate locations of the surfactant. Modified from Rosen (2004) .....	22
8. Structure of an extended surfactant molecule (Witthayapanyanon et al., 2006).....	23
9. Schematic of a system with oil, a surfactant and water with a lipophilic linker (Uchiyama et al., 2000). ....	24
10. Synergism and negative synergism in surface tension reduction effectiveness: (a) Pure surfactant 1, (b) and (c) mixture of surfactants 1 and 2 in different mole fraction and (d) Pure surfactant 2 (Rosen, 2004) .....	26
11. Schematic diagram of laboratory-based pilot scale processing of peanut and canola oil extraction. Solid line (—): surfactant wash step; Dashed line (- -): DI wash step (Do and Sabatini, 2011).....	27

12. Selected products at different stages of ASE and DI washing at optimum conditions of peanut and canola: (a) peanut and canola flours, (b) liquid fraction from L=L centrifuge of surfactant wash step, (c) liquid fraction from L=L centrifuge of DI washing step, and (d) free crude oil recovered from L=L centrifuge (Do and Sabatini, 2011) .....	28
13. Picture of (a) canola seeds and (b) a canola plant (NCCRP, 2011) .....	32
14. Biodiesel concept: from farm to fuel. <a href="http://farmwars.info/wp-content/uploads/2010/07/farm2fuel.gif">http://farmwars.info/wp-content/uploads/2010/07/farm2fuel.gif</a> .....	33
15. Transesterification reaction (Van Gerpen and Knothe, 2004).....	34
16. Free fatty acid reacts with catalyst forming soap and water (Van Gerpen and Knothe, 2004).....	34
17. Free fatty acid reduction by an acid-catalyzed pretreatment (Van Gerpen and Knothe, 2004).....	34
18. Diagram of aqueous surfactant-assisted extraction in Paper 1 .....	50
19. Dynamic IFT between 1 wt% anionic surfactant solution (AOT, AMA and SDS) and canola oil. Means within the bar graph followed by the same letter are not significantly different at $P \leq 0.05$ .....	53
20. Dynamic IFT of canola oil with mixed solution comprised of 0.03 M SDS and 0.01 M LS-series. Means within the bar graph followed by the same letter are not significantly different at $P \leq 0.05$ .....	54
21. Effect of NaCl concentration on dynamic IFT of each surfactant system with canola oil.....	56
22. Surfactant of 0.2 M NaCl with (a) 0.03 M SDS and (b) 0.03 M SDS plus 0.01 M LS-1.....	56
23. Scatter plot of dynamic IFT and oil detachment efficiency at various NaCl concentrations of canola oil with 0.03 M SDS. ....	58
24. Diagram of aqueous surfactant-assisted extraction in Paper 2 .....	70

25. Diagram of triple extraction with three stages. FS was fresh SDS solution and LF was liquid fraction. ....	74
26. Effect of SDS concentration on oil detachment and oil extraction efficiency at 20°C with solid-liquid ratio 1 g : 10 mL, stirring for 30 min. Means within the bar graph followed by the same color and letter are not significantly different at $P \leq 0.05$ . ....	78
27. Effect of extraction time on oil detachment and oil extraction efficiency for 0.02 M SDS solution at 20°C with solid-liquid ratio 1 g : 10 mL when mixing with (a) a magnetic stirrer and (b) an ultrasonication bath. Means within the bar graph of both (a) and (b) followed by the same color and letter are not significantly different at $P \leq 0.05$ . ....	79
28. Oil detachment efficiency versus extraction temperature using water, 0.02 M SDS solution and <i>n</i> -hexane as extraction medium with solid-liquid ratio 1 g : 10 mL, stirring for 45 min. Canola particle size was larger than 0.425 mm. Means within the bar graph followed by the same letter are not significantly different at $P \leq 0.05$ . ....	80
29. Impact of solid-liquid ratio on oil detachment and oil extraction efficiency for 0.02 M SDS solution at 20°C, stirring for 45 min. Means within the bar graph followed by the same color and letter are not significantly different at $P \leq 0.05$ . ....	81
30. Impact of triple extraction with three stages on oil detachment efficiency for 0.02 M SDS solution at 20°C, stirring for total contact time 45 min. Means within the bar graph followed by the same letter are not significantly different at $P \leq 0.05$ . ....	83
31. Impact of triple extraction with three stages on oil extraction efficiency for 0.02 M SDS solution at 20°C, stirring for total contact time 45 min. Means within the bar graph followed by the same letter are not significantly different at $P \leq 0.05$ . ....	83
32. Diagram of scaled-up ASE process. ....	84
33. Canola biodiesel (upper phase) and glycerol (lower phase) from three extracted oils and commercial oil ....	89

## LIST OF ABBREVIATIONS

IFT	.....	Interfacial Tension (mN/m)
CMC	.....	Critical Micelle Concentration
HLB	.....	Hydrophile-Lipophile Balance
S*	.....	Optimum Salinity
OD	.....	Oil Detachment Efficiency
OE	.....	Oil Extraction Efficiency
3E3S	.....	Triple Extraction with Three Stages

## LIST OF APPENDIX TABLES

<u>Table</u>	<u>Page</u>
A1. Surfactant properties and structure. ....	100
A2. Dynamic IFT of three commercial anionic surfactants and canola oil. ....	101
A3. Dynamic IFT of mixed surfactant systems and canola oil.....	101
A4. Dynamic IFT of SDS solution with canola oil at various NaCl concentrations.....	102
A5. Dynamic IFT of SDS and LS-1 solution with canola oil at various NaCl concentrations.....	104
A6. Dynamic IFT of SDS and LS-2 solution with canola oil at various NaCl concentrations.....	105
A7. Dynamic IFT of SDS and LS-3 solution with canola oil at various NaCl concentrations.....	106
A8. Dynamic IFT and oil detachment efficiency (OD) of ASE canola oil extraction using 0.03M SDS solution with various NaCl concentrations .....	107
B1. ASE conditions of each experiment in Paper 2. ....	108
B2. Oil detachment (OD) and oil extraction (OE) efficiency of ASE method with various SDS concentrations (Experiment 1, Paper 2). ....	109
B3. Oil detachment (OD) and oil extraction (OE) efficiency of ASE method using magnetic stirrer and ultrasonication bath with various extraction times (Experiment 2, Paper 2) .....	110
B4. Oil detachment (OD) and oil extraction (OE) efficiency of ASE method using extraction combination between ultrasonication and stirring with various extraction time ratios (Experiment 2, Paper 2). ....	111

B5. Oil detachment (OD) efficiency at 20°C of ASE method oil using water, 0.02 M SDS and <i>n</i> -hexane as extraction medium (Experiment 3, Paper 2). .....	112
B6. Oil detachment (OD) efficiency of ASE method using water and 0.02M SDS solution as extraction medium with various extraction temperatures (Experiment 3, Paper 2). .....	113
B7. Oil detachment (OD) and oil extraction (OE) efficiency of ASE method with various solid-liquid ratios (Experiment 4, Paper 2). .....	114
B8. Oil detachment (OD) and oil extraction (OE) efficiency of ASE method using triple extraction with three stages (3E3S) with various solid-liquid ratios (Experiment 5, Paper 2). .....	115
C1. Fatty acid profile of three extracted canola oils and commercial canola oil. ....	116
C2. Water content and acid value of extracted canola oil and commercial canola oil. ....	116
C3. Water and soap content of canola biodiesel from three extracted oils and commercial oil. ....	117
C4. Kinematic viscosity and cloud point of canola biodiesel from three extracted oils and commercial oil. ....	118
C5. Acid value and oxidative stability of canola biodiesel from three extracted oils and commercial oil. ....	119

## CHAPTER 1. GENERAL INTRODUCTION

### 1.1. Introduction

Due to depletion of fossil oil and concerns about environmental quality, many alternative liquid fuels have been developed. Among them, biodiesel is one of the interesting liquid fuels which has the potential to increase the world's energy security and reduces carbon dioxide emissions. Vegetable oil is the main biodiesel feedstock due to its availability. Vegetable oil is converted to biodiesel by the transesterification reaction. The performance of this process depends on several factors including type and concentration of alcohol and catalyst, operating temperature and reaction time, and also the quality of the vegetable oil (Van Gerpen and Knothe, 2004). The contents of free fatty acids, water, and phospholipids are three important parameters for biodiesel feedstock quality. A high content of free fatty acids (high acid value) and water can reduce biodiesel yield and quality by contributing to soap formation. Presence of phospholipids has the potential to clog diesel engines. Many refining steps are required to make vegetable oil suitable for conversion to biodiesel.

Apart from the quality of feedstock concern, there is a growing concern about the vegetable oil extraction process. Hexane is broadly used in the oilseed extraction industry because of its high oil extraction efficiency, >95% (Beckel, Belter and Smith, 1946). However, hexane is a volatile organic compound that has been categorized as a hazardous air pollutant and has a strict emission standard (100 mg/Nm<sup>3</sup>). For every one ton of oilseed processed, there is an hexane loss between 0.7-4.5 L (U.S. Environmental Protection Agency (EPA), 2001). Even though the current technology passes the hexane

emission standard, workers who have been exposed to hexane at 15 ppm/day for 3 months show peripheral nerve damage (Do and Sabatini, 2010). Moreover, 0.8 mg/kg hexane is typically found in refined vegetable oil (Swanson, 2012). Thus, it is a requirement to fully contain hexane, and have a leak detection system and good solvent evaporation processes. This strict emission standard provides a strong incentive to develop a hexane-free vegetable oil extraction method.

Several solvents have been evaluated to replace hexane in a vegetable oil extraction process such as: water (Hanmoungjai, Pyle and Niranjan, 2000), ethanol (Kwiatkowski and Cheryan, 2002), acetone (Youngs and Sallans, 1955), heptanes and isohexane (Wan et al., 1995), isohexane with alcohol mixture (Kuk and Hron, 1998), mixed propane with supercritical fluid CO<sub>2</sub> (Pederssetti et al., 2011) and even vegetable oil itself (Strop and Perry, 1989).

An aqueous extraction process (AEP), which uses water as the extraction medium, is one potential vegetable oil extraction method. Currently this method can achieve >70% oil extraction efficiency (Rosenthal, Pyle and Niranjan, 1996). However, to achieve this yield, the extractor must be operated at 50°C and a large amount of chemical reagent applied to adjust the pH to 12 (Rosenthal, Pyle and Niranjan, 1998).

One way to enhance AEP is to incorporate enzymes such as cellulase to hydrolyze plant cell walls, as well as protease to hydrolyze the protein network and assist demulsification. Since enzymes are selective to specific classes of compounds, a combination of enzymes is advantageous. This multiple enzyme-assisted aqueous extraction yielded 65 to 75% oil (Dominguez, Ndfiez and Lema, 1994; Latif, Diosady

and Anwar, 2008; Rosenthal et al., 1996). However, use of multiple enzymes further complicates optimization of reaction conditions such as pH and temperature. Recently, the yield of oil was increased to > 90% by applying flaking and extrusion as mechanical pre-treatments with use of a single proteolytic enzyme (de Moura et al., 2008; de Moura and Johnson, 2009; Lamsal and Johnson, 2007; Lamsal, Murphy and Johnson, 2006). These pre-treatments disrupt cell walls, which is a key to recovering oil (Rosenthal et al., 1996); thus, a cellulase enzyme is not necessary.

Another way to enhance AEP is to incorporate a surfactant, resulting in an operation known as aqueous surfactant-assisted extraction or “ASE”. The main concept of this method is to use surfactant solution to reduce interfacial tension (IFT) between vegetable oil and extraction media. By definition, IFT is the repulsive energy between two different species of molecules (Rosen, 2004). It is the minimum energy (heat, gravity and shear force) required to make one phase disperse into another phase, increasing the surface area between two different phases. The high IFT implies that these two species of molecules are very different. In the case of water and vegetable oil, the IFT value is between 20 and 30 mN/m (Gunstone, 2004). Surfactants can minimize the IFT of the system and help vegetable oil easily detach from the oilseed surface. Decreased IFT, moreover, results in reduced size of oil droplets, helping them disperse into extraction media (Do and Sabatini, 2010).

The ASE method achieved an oil efficiency of more than 80% without thermal energy in laboratory bench scale (Do and Sabatini, 2010; Kadioglu, Phan and Sabatini, 2011; Naksuk, Sabatini and Tongcumpou, 2009). The extracted oil also had a lower

content of free fatty acids and phospholipids, so the refinery cost of vegetable oil is reduced. Furthermore, ASE eliminates the costs of installing and maintaining a hexane-leak detection system and reduces risk to workers. Absence of toxic hexane solvent also gives superior co-product meal quality (Rosenthal et al., 1996). Several literature reports suggest that the IFT should be reduced to 0.01 mN/m (ultralow level) for this extraction method (Do and Sabatini, 2010; Kadioglu et al., 2011; Naksuk et al., 2009). A commercial surfactant, alone, cannot reduce IFT to this ultralow level. A co-surfactant such as a short chain alcohol (Sabatini, Acosta and Harwell, 2003) or extended surfactant is required (Do and Sabatini, 2010; Witthayapanyanon et al., 2006). Extended surfactants are a new type of surfactant, in which propoxylate and/or ethoxylate groups are inserted between polar and non-polar parts of the molecule. These groups allow the surfactant molecule to stretch out further into the oil and water phase, resulting in a reduction of IFT of the system to ultralow level (Witthayapanyanon et al., 2006).

However, using a volatile and flammable co-surfactant requires a leak proof system; and the extended surfactant is still not commercialized. Moreover, most of the research on ASE thus far has been conducted at the laboratory bench level (2-5 g oilseed) which is insufficient to evaluate the quality of biodiesel produced from the oil. To the best of our knowledge, there has been no work investigating the property of biodiesel from this ASE extracted oil.

## **1.2. Objectives of the Study**

The main objectives of this work were:

(1) selecting and formulating an inexpensive commercial surfactant system as the extraction medium in the ASE method of canola oil extraction based on the IFT value;

(2) maximizing the oil recovery of the ASE method by modification of the extraction conditions;

(3) scaling up the ASE process to extract oil from 300 g ground canola per batch for quality analysis and conversion to biodiesel; and

(4) evaluating the biodiesel product quality to demonstrate the potential use of the extracted oil from the ASE method as a biodiesel feedstock following biodiesel standard ASTM D6751 (American Society for Testing and Materials, 2009).

## **1.3. Thesis Organization**

This thesis consists of a literature review and two research papers. The literature review provided information regarding current technology of vegetable oil extraction, ASE method, canola and biodiesel.

Paper 1, entitled “Improving Canola Oil Detachment Efficiency by Reducing Interfacial Tension,” detailed the surfactant system selection based on the dynamic IFT value with canola oil, improved the dynamic IFT reduction techniques, and investigated the correlation between the dynamic IFT and canola oil detachment efficiency in the ASE

method. Consequently, an inexpensive commercial surfactant system was formulated (objective 1) and applied to the ASE process for canola oil extraction in Paper 2.

Paper 2, entitled “Development and Scale up of Aqueous Surfactant-assisted Extraction of Canola oil for Use as Biodiesel Feedstock,” detailed the effect of extraction conditions on oil extraction and oil detachment efficiency through the ASE method; scaled up the ASE process; and compared important quality characteristics of biodiesel from ASE, screw press, Soxhlet hexane extraction and commercial canola oil. The highest oil detachment efficiency was 80% in the absence of hexane at room temperature (objectives 2 and 3). The extracted oil had a good biodiesel feedstock quality; and the biodiesel product passed the ASTM D6571 biodiesel standard (objective 4).

Since the author will be awarded a double degree in Master of Science scholarship, which is through the collaboration between the Environmental and Conservation Sciences program of North Dakota State University and the Environmental and Hazardous Waste Management program of Chulalongkorn University (Thailand), the information in paper 1 and the effect of extraction conditions on oil detachment and oil extraction efficiency in paper 2 were also included in the thesis submitted to Chulalongkorn University.

#### **1.4. References**

American Society for Testing and Materials. 2009. "Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels." ASTM D6751-09. West Conshohocken: ASTM.

- Beckel, A. C., Belter, P. A. and Smith, A. K. 1946. Laboratory study of continuous vegetable oil extraction: countercurrent extractor, rising-film evaporator, and oil stripper. Industrial and Engineering Chemistry 18(1): 56-58.
- de Moura, J. M. L. N., Campbell, K. A., Mahfuz, A., Jung, S., Glatz, C. E. and Johnson, L. 2008. Enzyme-assisted aqueous extraction of oil and protein from soybeans and cream de-emulsification. Journal of American Oil Chemists' Society 85: 985-995.
- de Moura, J. M. L. N. and Johnson, L. A. 2009. Two-stage countercurrent enzyme-assisted aqueous extraction processing of oil and protein from soybeans. Journal of American Oil Chemists' Society 86: 283-289.
- Do, L. D. and Sabatini, D. A. 2010. Aqueous extended-surfactant based method for vegetable oil extraction: proof of concept. Journal of American Oil Chemists' Society 87: 1211-1220.
- Dominguez, H., Ndfiez, M. J. and Lema, J. M. 1994. Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: a review. Journal of Food Chemistry 49: 271-286.
- Gunstone, F. D. 2004. Rapeseed and canola oil: production, processing, properties and uses. Florida: CRS Press.
- Hanmoungjai, P., Pyle, L. and Niranjana, K. 2000. Extraction of rice bran oil using aqueous media. Journal of Chemical Technology and Biotechnology 75: 348-352.
- Kadioglu, S. I., Phan, T. T. and Sabatini, D. A. 2011. Surfactant-based oil extraction of corn germ. Journal of American Oil Chemists' Society 88: 863-869.

- Kuk, M. S. and Hron, R. J. 1998. Cottonseed extraction with a new solvent system: isohexane and alcohol mixtures. Journal of American Oil Chemists' Society 75: 927-930.
- Kwiatkowski, J. R. and Cheryan, M. 2002. Extraction of oil from ground corn using ethanol. Journal of American Oil Chemists' Society 79: 825-830.
- Lamsal, B. P. and Johnson, L. A. 2007. Separating oil from aqueous extraction fractions of soybean. Journal of American Oil Chemists' Society 84: 785-792.
- Lamsal, B. P., Murphy, P. A. and Johnson, L. A. 2006. Flaking and extrusion as mechanical treatments for enzyme-assisted aqueous extraction of oil from soybeans. Journal of American Oil Chemists' Society 83: 973-979.
- Latif, S., Diosady, L. L. and Anwar, F. 2008. Enzyme-assisted aqueous extraction of oil and protein from canola (*Brassica napus* L.) seeds. European Journal of Lipid Science and Technology 110: 887-892.
- Naksuk, A., Sabatini, D. A. and Tongcumpou, C. 2009. Microemulsion-based palm kernel oil extraction using mixed surfactant solutions. Industrial Crops and Products 30: 194-198.
- Pederssetti, M. M., Palú, F., Silva, E. A. d., Rohling, J. H., Cardozo-Filho, L. and Dariva, C. 2011. Extraction of canola seed (*Brassica napus*) oil using compressed propane and supercritical carbon dioxide. Journal of Food Engineering 102: 189-196.
- Rosen, M. J. 2004. Surfactant and Interfacial Phenomena. 2nd ed. New York: John Wiley & Sons.

- Rosenthal, A., Pyle, D. L. and Niranjana, K. 1996. Aqueous and enzymatic process for edible oil extraction. Enzyme and Microbial Technology 19: 402-420.
- Rosenthal, A., Pyle, D. L. and Niranjana, K. 1998. Simultaneous aqueous extraction of oil and protein from soybean: Mechanisms for process design. Transaction of Institution of Chemical Engineers 76(C): 224-230.
- Sabatini, D. A., Acosta, E. and Harwell, J. H. 2003. Linker molecules in surfactant mixtures. Current Opinion in Colloid and Interface Science 8: 316-326.
- Strop, H. R. and Perry, R. R. 1989. "Vegetable oil extraction process." USA. Vol. 4,808,426.
- Swanson, B. G. Hexane extraction in soyfood processing. [Online]. 2012. Available from: <http://www.soyfoods.org/wp-content/uploads/Regulatory%20Expert%20Document-Barry%20Swanson%20revised.pdf> [20 June 2012]
- U.S. Environmental Protection Agency (EPA). 2001. "National emission standards for hazardous air pollutants." Solvent extraction for vegetable oil production. Washington, DC: EPA.
- Van Gerpen, J. and Knothe, G. Basic of the transesterification reaction. 2004. In G. Knothe, J. Krahl and J. Van Gerpen (eds.), The biodiesel handbook, pp.26-41. Champaign: AOCS Press.
- Wan, P. J., Hron, R. J., Dowd, M. K., Kuk, M. S. and Conkerton, E. J. 1995. Alternative hydrocarbon solvents for cottonseed extraction: plant trials. Journal of American Oil Chemists' Society 72(6): 661-664.

Witthayapanyanon, A., Acosta, E. J., Harwell, J. H. and Sabatini, D. A. 2006.

Formulation of ultralow interfacial tension systems using extended surfactants.

Journal of Surfactants and Detergents 9: 331-339.

Youngs, C. G. and Sallans, H. R. 1955. Acetone as a selective solvent for vegetable oils.

Journal of American Oil Chemists' Society 32(7): 397-400.

## CHAPTER 2. LITERATURE REVIEW

### 2.1. Technology and Solvent for Oilseed Extraction

Over five decades, hexane and alkanes with similar boiling points have been used as oilseed extraction solvents in most of the oilseed industries. Due to its economical efficiency and superior solvent characteristics, no alternative solvent has been deemed suitable for replacing hexane. However, disadvantages to using hexane still remain that make it not the ideal solvent, such as its toxicological and environmental concerns, the leak detection system's high investment cost, and low quality meal destined for feed or food (Gregory and Horsman, 1997). Hexane is a volatile organic carbon (VOC) that has been categorized as a hazardous air pollutant and subjected to a strict emission standard (100 mg/Nm<sup>3</sup>). For every ton of oilseed, between 0.7-4.5 L of hexane is lost in the oil extraction process (U.S. Environmental Protection Agency (EPA), 2001). Even though the current technology allows for emissions that are in compliance with the hexane emission standard, workers who were exposed to 15 ppm of hexane per day for 3 months were shown to have suffered peripheral nerve damage (Do and Sabatini, 2010). Moreover, the amount of hexane typically found in refined vegetable oil is 0.8 mg/kg (Swanson, 2012). In the 1970s, there was strong interest in finding alternative solvents to replace hexane, due to economic reasons. At that time, the Organization of Petroleum Exporting Countries (OPEC) increased the price of petroleum and its derivatives six to eight times the previous cost. Thus, this situation increased the operating cost with hexane, a derivative of petroleum (Johnson, 1997).

Gregory & Horsman (1997) suggested that the joint involvement of industry and researchers in identifying an alternative solvent for oilseed extraction will enhance its credibility and acceptance. They provided the criteria to evaluate the efficiency of a solvent, as shown in Table 1. Finally, they highly recommended that full-scale tests were used to lend economic credibility to research involving alternative solvents. In this work, we also focused on scaling up our alternative extraction media and extraction processes.

### 2.1.1. Oilseed extraction processes

For thousands of years, humans have extracted the oil and fat from oilseeds, fruits, and fatty animal tissues. The oldest method for oil extraction is mechanical pressing, which usually requires seed pretreatment (Johnson, 1997). In the commercial vegetable oil extraction industry, many steps are involved in order to achieve the highest oil yield and quality (Figure 1). However, different oilseeds require different extraction processes, in addition to different methods of pretreatment (Table 2).

Table 1. Numerical evaluation of attributes of solvents for vegetable oil extraction (Gregory and Horsman, 1997).

Weighting Factor	Attribute	<i>n</i> -hexane		Isohexane		Isopropyl alcohol	
		ranking	score	ranking	score	ranking	score
20	Extraction Efficiency	3	60	2	40	1	20
20	Environmental Friendliness	1	20	2	40	3	60
15	Capital Requirements	3	45	2	30	1	15
10	Price Effectiveness	3	30	2	20	1	10
10	Toxicity	1	10	2	20	3	30
10	Flammability	1	10	2	20	3	30
5	Energy Efficiency	3	15	2	10	1	5
5	Meal Quality Enhancement	1	5	2	10	3	15
5	Oil Quality Enhancement	1	5	2	10	3	15
<b>100</b>	<b>Total</b>		<b>200</b>		<b>200</b>		<b>200</b>

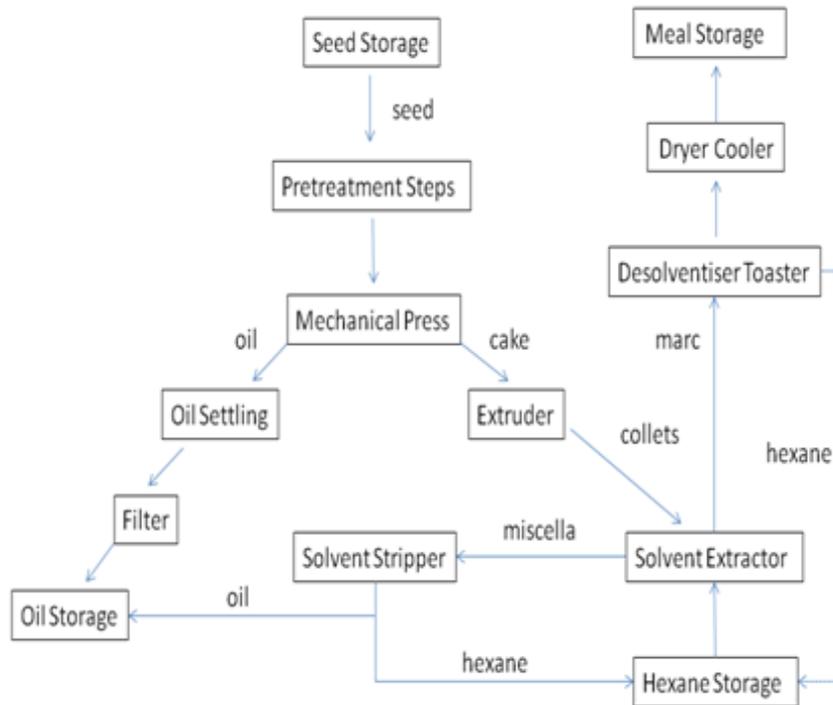


Figure 1. Conventional commercial vegetable oil extraction process (Booth, 2004).

Table 2. Oil content of various oilseeds with the prevalent method of extraction (Johnson, 1997).

Source	Oil Content (%)	Prevalent Method of Recovery
Soybean	19	Direct solvent extraction
Corn (germ)	40	Wet or dry milling and prepress solvent extraction
Tallow (edible)	70-95	Wet or dry rendering
Canola	42	Prepress solvent extraction
Coconut (dried copra)	66	Hard pressing
Cottonseed	19	Hard pressing, or prepress or direct solvent extraction
Lard	70-95	Wet or dry rendering
Palm	47	Hard pressing
Palm Kernel	48	Hard pressing
Sunflower	40	Prepress solvent extraction
Peanut (shelled)	47	Hard pressing or prepress solvent extraction

**2.1.1.1. Pretreatment steps.** Pretreatment or conditioning steps are applied to prepare oilseeds before extraction processes, as presented in Figure 1. These steps are required to increase the surface area, reduce the moisture content, and disrupt the oil cells within the seeds (Booth, 2004). Oilseeds are reduced in size by the grinding step. This step breaks down the oilseed cell structure, increasing the surface area and releasing the oil. The most suitable particle size for oilseed extraction is between 0.21-0.42 mm (Do and Sabatini, 2010; Kadioglu, Phan and Sabatini, 2011; Naksuk, Sabatini and Tongcumpou, 2009).

Furthermore, a thermal treatment, known as cooking, is used to heat the ground oilseeds to 75-85 °C. It breaks down the remaining oil cells and promotes the coalescing of small oil droplets to larger oil droplets. Oil viscosity is also reduced, making it easier for oil to be extracted by the subsequent processes (Booth, 2004). Some researchers developed this process to achieve great oil yield. For example, Li et al. (2011) reported that using an electric far-infrared oven (60 min at 112°C) was able to increase wheat germ oil enzymatic extraction to 84.82%, while a regular pressure cooker was able to extract 78.37%.

**2.1.1.2. Mechanical extraction.** Oilseeds which have an oil content more than 30 wt%, must be put through hard pressing or prepressing before solvent extraction. According to Khan and Hanna (1983), the mechanical press can be classified into three types: a wedge press, hydraulic press, and screw press. Although a press is able to disrupt and extract most of the oil, some of it is adsorbed on the surface and/or inside of the

oilseeds. In order to extract all of the oil, solvent extraction is applied to oilseed extraction processes.

Ultrasonication has been used for oilseed extraction. Ultrasonication is the application of ultrasonic (>20 kHz) waves in liquid media, which make the liquid sample move violently (agitation). The sound wave penetrates through the liquid media, generating high pressure (compression) and low pressure (rarefaction) cycles. In the rarefaction period, many small vacuum bubbles or voids are created in the liquid media by high intensity sonic waves. These voids collapse violently (cavitation) during compression. The implosion of the cavitation bubbles generates hydrodynamic shear-force. This force is very powerful, even the propeller of a warship can be eroded by it (David, 2002). Thus, ultrasonication is applied to reduce the size of oilseeds, and breakdown the cell walls. Shah et al. (2004) applied 10 min of ultrasonication for aqueous enzymatic jatropha oil extraction. They found that ultrasonication was able to increase the oil extraction efficiency from 67% to 74% and cut down the process time from 18 h to 6 h.

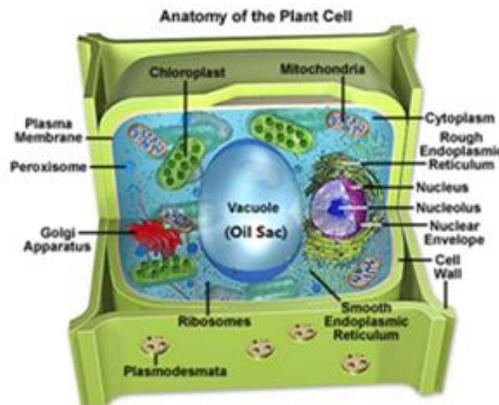


Figure 2. Anatomy of a plant cell: vegetable oil is contained in the vacuole (Davidson, 2011).

**2.1.1.3. Solvent extraction.** The process of solvent extraction is the mass transfer process that selectively transfers a compound from one phase to another phase in order to obtain the compound in a pure or enriched form. Hexane has been used in most commercial vegetable oil extraction plants for the past 50 years (Johnson, 1997). Hexane solvent extraction had been developed to the point where it was not necessary to apply the mechanical press. Figure 3 illustrates the Crown Model III from the Crown Iron Works Company. This extractor applies counter current flow to extract oilseeds with hexane. The bed of oil-bearing material should allow for solvent percolation. Therefore, seed particles should not be too fine. The oil from the fresh oilseeds was extracted by miscella (a mixture of vegetable oil and solvent) coming in from the recycling pumps as the counter current loop extraction. Compared with a batch extraction method, counter current extraction is accomplished with less solvent in the extraction process (Wisnborn et al., 1993).

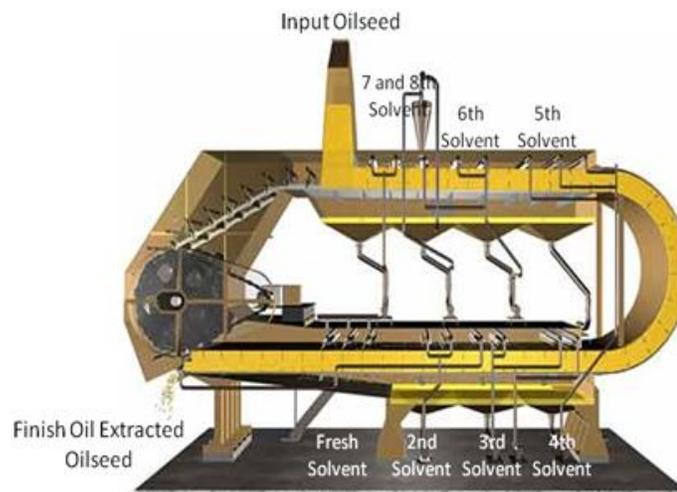


Figure 3. Crown Model III oilseed extractor.

[http://www.crowniron.com/technologies/ext\\_model3.cfm](http://www.crowniron.com/technologies/ext_model3.cfm).

However, as mentioned previously, hexane is not the ideal solvent. Recently many alternative solvents and extraction media have been developed. One promising extraction medium is a surfactant solution, used in aqueous surfactant-assisted extraction.

## **2.2. Aqueous Surfactant-Assisted Extraction (ASE)**

A surfactant is an amphipathic substance that has both a hydrophobic part (tail group) and hydrophilic part (head group) as shown in Figure 4. Due to having both hydrophobic and hydrophilic parts, a surfactant has a unique characteristic that makes it suitable to be used as an extraction medium. A surfactant can dissolve both non-polar compounds and polar compounds by reducing the interfacial tension (IFT) of the system. By definition, IFT is the repulsive energy between two different species of molecules (Rosen, 2004). In other words, it is the minimum energy (heat, gravity and shear force) that is required to make one phase disperse into another phase, increasing the surface area between two different phases. A high IFT value implies that the two molecules are very different in structure, such as vegetable oil (non-polar compound) and water (polar compound) with an IFT value between 20 and 30 mN/m (Gunstone, 2004). Thus, water and oil cannot be homogeneously mixed and separated into two immiscible phases. Once a surfactant is introduced into this system, the IFT of system is reduced and then the two immiscible phases can be dispersed in each other, forming an emulsion. Therefore, a surfactant plays a role as a linker between the vegetable oil and water. In conclusion, the main concept of ASE is reducing the IFT of the system to detach oil from oilseeds. Do and Sabatini (2010) suggested that the IFT should be reduced to an ultralow level ( $<0.01$  mN/m) to break up the oil droplets, and release the oil from the disrupted cells.

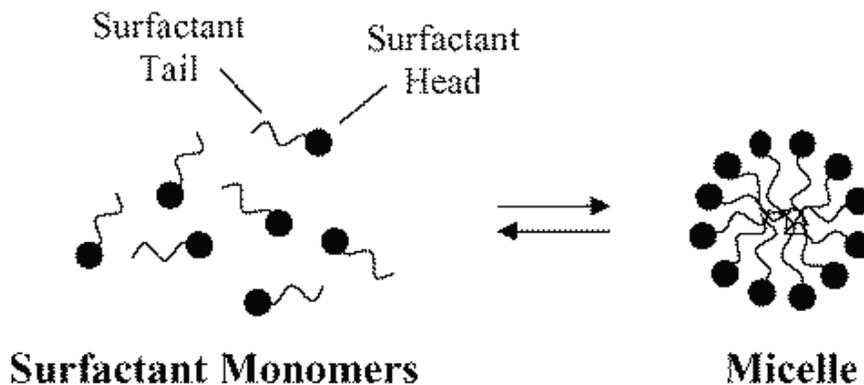


Figure 4. Surfactant structure (Carlota et al., 2005).

### 2.2.1. How to reduce interfacial tension

As mentioned above, IFT is repulsive energy between two different molecules. In Figure 5, at the interface of two different pure liquid phases “a” and “b”, there is potential energy between different molecules that is always greater than that of similar molecules in the interior of its bulk phase. The net potential energy of the “a” molecule at the interface over those in the interior of that phase can be presented as  $(A_{aa} - A_{ab})$ , where  $A_{aa}$  and  $A_{ab}$  represent the molecule interaction energy between molecule “a” and “a”, and “a” and “b”, respectively. Similarly, the net potential energy of “b” molecules at the interface over that in the interior is  $(A_{bb} - A_{ab})$ , where  $A_{bb}$  and  $A_{ab}$  represent the molecule interaction energy between molecule “b” and “b”, and “a” and “b”, respectively. Thus, the total net potential energy at the interface over that in the interior of the bulk phase is  $(A_{aa} - A_{ab}) + (A_{bb} - A_{ab})$  or  $A_{aa} + A_{bb} - 2A_{ab}$ , named interfacial free energy. This is the minimum work required to create the interface, separating two different species of molecules into two immiscible phases.

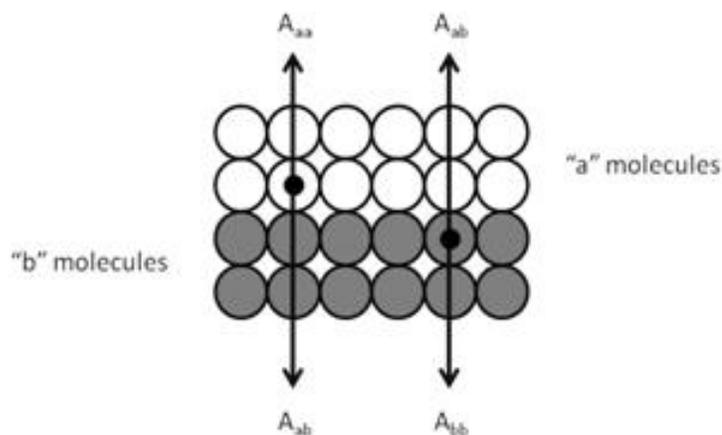


Figure 5. Molecule interaction energy at the interface between two pure liquid phases “a” and “b” (Rosen, 2004).

In order to minimize this repulsive energy, the interaction energy between different molecules,  $A_{ab}$ , must be large; in other words, molecule “a” and “b” must be similar in nature to each other (polar and polar compounds or nonpolar and nonpolar compounds). Once surfactants are introduced into the two immiscible phases, they are absorbed at the interface between two immiscible phases. A surfactant will orient its hydrophilic head group toward the polar solvent and its hydrophobic tail group toward the nonpolar solvent, in accordance with the “like dissolves like” concept. This phenomenon produces a very large  $A_{ab}$  and therefore decreases the IFT of the system. There are two rules of thumb for using a surfactant to reduce the IFT, which is described in more detail below. First, the concentration of the surfactant should reach its critical micelle concentration (CMC). Second, the Winsor R ratio (i.e., the ratio of the interaction energy within the hydrophobic part and hydrophilic part of the system) should be equal to one and have very high interaction energy in both the hydrophobic and hydrophilic parts.

**2.2.1.1. Critical micelle concentration.** Critical micelle concentration (CMC) is the concentration of the surfactant when the first micelle is formed (Figure 6). At this concentration, surfactants are adsorbed on the entire interface area between two immiscible phases. Thus, it provides the largest  $A_{ab}$  to the system, resulting in the lowest IFT. Moreover, Witthayapanyanon et al. (2006) reported that an extended surfactant was able to reduce IFT to lower than its CMC level once the surfactant concentration was increased to the critical microemulsion concentration ( $C_{\mu C}$ ).

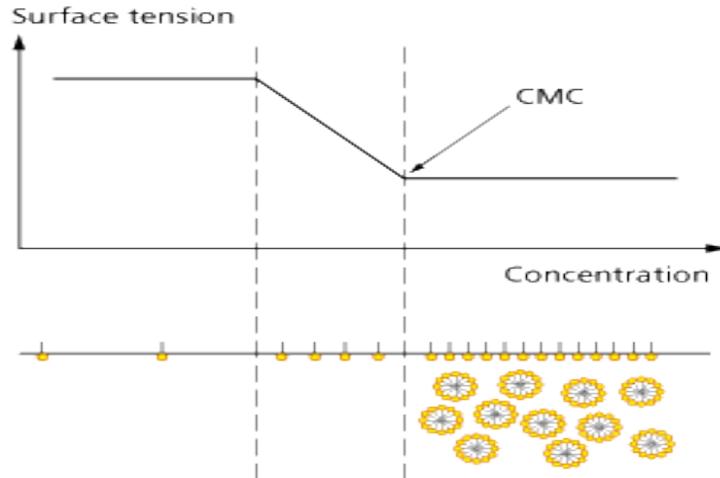


Figure 6. Effect of surfactant concentration on surface tension or interfacial tension (Kunjappu, 2003).

**2.2.1.2. Winsor R ratio.** The Winsor R ratio is used to interpret changes in the hydrophilic solvent (W), the lipophilic solvent (O), and the surfactant (S) to the IFT and phase volume in terms of the molecular interaction involved (Rosen, 2004).

$$R = \frac{Net A_{SO}}{Net A_{SW}} = \frac{A_{SO} - A_{OO} - A_{LL}}{A_{SW} - A_{WW} - A_{HH}} \quad (\text{Equation 1})$$

where Net  $A_{SO}$  is the net interaction between the surfactant and oil (hydrophobic), Net  $A_{SW}$  is the net interaction between the surfactant and water (hydrophilic),  $A_{SO}$  is the interaction between the surfactant and oil,  $A_{SW}$  is the interaction between the surfactant and water,  $A_{OO}$  is the interaction energy among oil molecules,  $A_{LL}$  is the interaction among the tails of the surfactant molecules,  $A_{WW}$  is the interaction energy among the water molecules, and  $A_{HH}$  is the interaction among the surfactant heads (Witthayapanyanon et al., 2006).

In order to minimize IFT, the Winsor R ratio should equal one ( $R=1$ ) and have very high interaction energy in both the hydrophobic and hydrophilic parts. In other words, Net  $A_{SO}$  and Net  $A_{SW}$  must be high and very close to each other. There are several methods that can modify this ratio.

**2.2.1.2.1. Electrolyte concentration and temperature.** Different types of surfactants have different effects on the electrolyte concentration and temperature. Basically, surfactants are divided into four types, based on the charge of the polar part of the surfactant structure: anionic surfactants, cationic surfactants, nonionic surfactants and zwitterionic surfactants (which have both positive and negative charges). Introducing electrolytes to the system has an impact on the Net  $A_{SO}$  and Net  $A_{SW}$  of the ionic surfactant by reducing  $A_{SW}$  and increasing  $A_{SO}$ . Electrolytes, such as NaCl, minimize the electrical interaction of the ionic head group of the surfactant, causing closer packing. This can cause changes in the micellar structure from its normal spherical shape to a reverse micelle formation. In the other words, the surfactant is changed from its hydrophilic form to its hydrophobic form. The NaCl concentration that provides the

lowest IFT is called optimum salinity ( $S^*$ ). Unlike an ionic surfactant, the electrolyte concentration marginally affects a nonionic surfactant. In order to modify the Winsor R ratio of a nonionic surfactant system, temperature is applied. Increasing temperature can cause the dehydration of the POE chains in the nonionic surfactant structure, making the surfactant more hydrophobic (Rosen, 2004). Figure 7 shows the effects of molecular environmental conditions on interfacial tension and phase volumes. Introducing an electrolyte (NaCl) has been the most common method to reducing IFT, and its application together with other methods has been studied by a number of researches (Do and Sabatini, 2010; Naksuk et al., 2009; Tongcumpou et al., 2006; Witthayapanyanon et al., 2006).

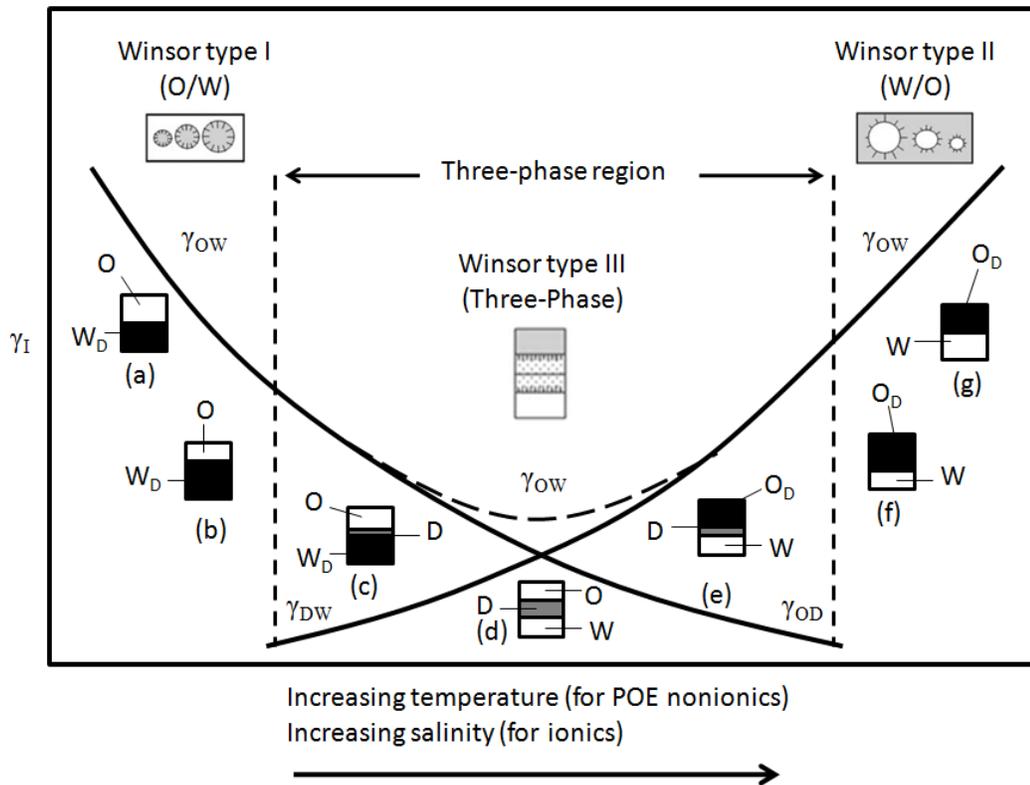


Figure 7. Effect of molecular environmental conditions on interfacial tension and phase volumes. Shaded phases indicate locations of the surfactant. Modified from Rosen (2004).

**2.2.1.2.2. Extended surfactant.** An extended surfactant is a surfactant in which groups of propyleneoxide (PO) and/or ethyleneoxide (EO) are inserted between the hydrophobic and hydrophilic parts (Figure 8). These groups make the surfactant stretch out further into both of oil and water phases, increasing the adsorption of the surfactant in the interfacial region and reducing the IFT of the system. Witthayapanyanon et al. (2006) applied an extended surfactant,  $C_{14-15}-(PO)_8-SO_4Na$  (0.2 %wt), to reduce the IFT of various of vegetable oils. This extended surfactant reduced the IFT to an ultralow level ( $<0.01$  mN/m) at optimum salinity. Moreover, Do and Sabatini (2010) used various kinds of extended surfactants, and varied the numbers of PO and EO groups, with triolein (representative as a main component of vegetable oil) and achieved ultralow IFT.

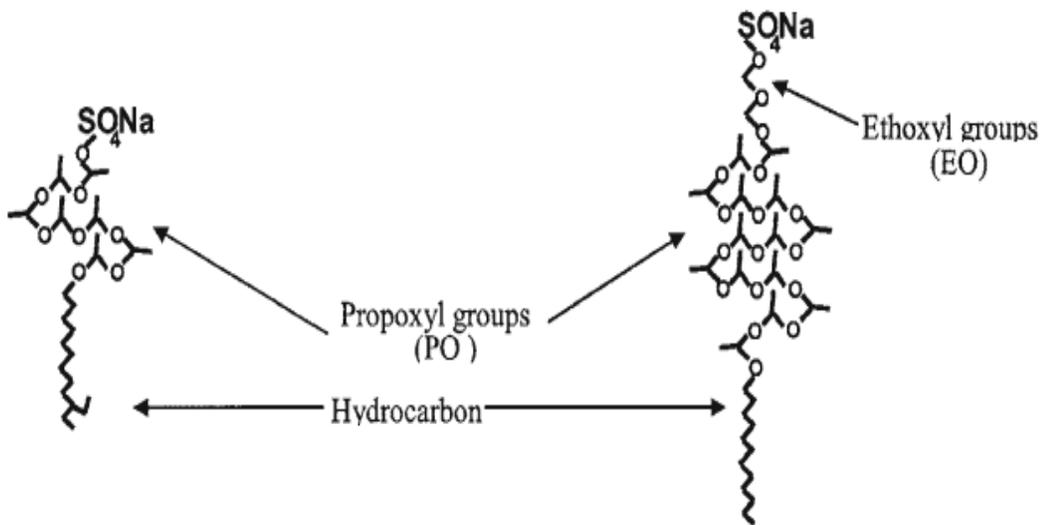


Figure 8. Structure of an extended surfactant molecule (Witthayapanyanon et al., 2006).

**2.2.1.2.3. Linker (co-surfactant).** A linker, known as a co-surfactant or co-solvent, is one kind of an amphipathic substance that can reduce the IFT of a system. However, it cannot form a micellar structure, an example of a linker is n-alkyl alcohol

(Rosen, 2004). Linkers can be classified into two main types, lipophilic and hydrophilic linkers. A lipophilic linker is a linker with a molecule that is mainly lipophilic, examples of which include medium and long chain alcohols. A lipophilic linker thus interacts with both the lipophilic part of a surfactant molecule (tail group) and the non-polar phase (vegetable oil). Unlike a lipophilic, a hydrophilic linker is mainly hydrophilic; a short chain alcohol is an example of a lipophilic linker. It is used to link the hydrophilic part of the surfactant molecule (head group) and the polar phase (water). Thus, introducing these linkers into a system increases the similarity among the vegetable oil, surfactant, and water, reducing the IFT of the system. Sabatini et al. (2003) and Uchiyama et al. (2000) recommended the combination of lipophilic and hydrophilic linkers, because the resulting solubilization of the non-polar phase and surfactant solution lowers the IFT value of a system.

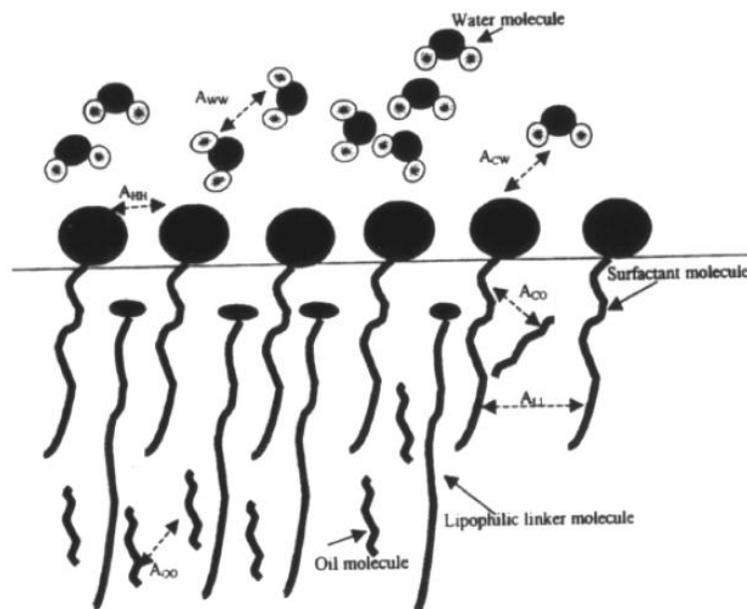


Figure 9. Schematic of a system with oil, a surfactant and water with a lipophilic linker (Uchiyama et al., 2000).

**2.2.1.2.4. Synergism effect.** Synergism happens when two surfactants are mixed and generate better properties (a lower CMC level, surface tension and interfacial tension) than those produced by the individual surfactants (Figure 10b). This phenomenon occurs due to electrostatic forces that affect the interaction of two surfactants. As a result, the strength of their attractive interactions decreases in the following order: anionic-cationic > anionic-zwitterionic > ionic-nonionic > nonionic-nonionic. In general, an increase in the electrolyte concentration of the aqueous phase produces a decrease in the attractive interaction. However, in the case of an anionic-nonionic mixture, it increases the attractive interaction. It creates a complex formation between the  $\text{Na}^+$  and the ether oxygen of the POE chain, resulting in its acquisition of a positive charge, which increases the strength of its interaction with the anionic surfactant (Rosen, 2004). Klongklaew et al. (2005) used a mixed surfactant of an anionic and a nonionic surfactant to reduce the IFT of a soybean oil and surfactant solution. Naksuk et al. (2009) also applied this synergism phenomenon, using an anionic-nonionic mixed surfactant, to minimize the IFT of a palm kernel oil and surfactant system.

Even though the mixture of two surfactants can produce better properties, these two surfactants must be mixed together at a suitable ratio. If the ratio of the surfactants is not suitable, it creates worse properties (higher CMC, surface tension and interfacial tension). This phenomenon is called negative synergism (Figure 10c).

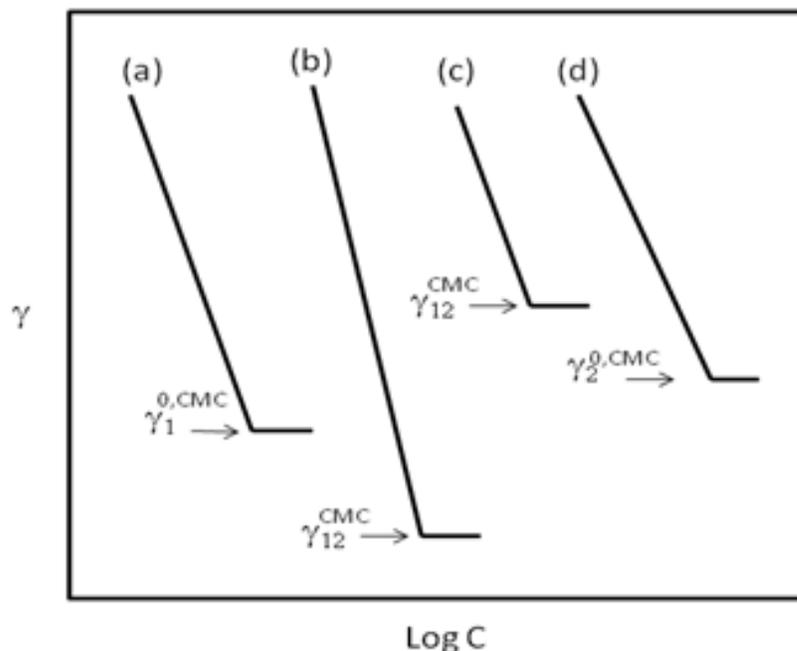


Figure 10. Synergism and negative synergism in surface tension reduction effectiveness: (a) pure surfactant 1, (b) and (c) mixture of surfactants 1 and 2 in different mole fractions and (d) pure surfactant 2 (Rosen, 2004).

### 2.2.2. Extraction process

Several research studies have focused on the possibility of using the ASE method to extract vegetable oil and protein. However, most of the research has been conducted at lab-bench level, extracting 2-5 g of vegetable oil. Tables 3 and 4 summarize the extraction process, oil extraction efficiency and extracted oil quality results from three studies. Do and Sabatini (2011) scaled up this process to the semi-continuous pilot scale (Figures 11 and 12). They applied the same conditions as they did their previous study (Do and Sabatini, 2010), but added a deionized water washing step as the second extraction process on 150 g of peanuts and canola seeds. The oil extraction efficiencies were 87.1% for peanuts and 85.6% for canola seeds.

One problem of the ASE method is emulsion formation. A demulsification process is required to break down the emulsion, resulting in an increase in oil recovery. A demulsification agent, a thermal treatment, an enzymatic treatment and centrifugation have been typically used in demulsification processes of the food industry (Chabrand et al., 2008; Rosenthal, Pyle and Niranjana, 1996). In the petroleum industry a high voltage technique was used to demulsify a petroleum emulsion from an oil recovery process (Wang, Lee and Chan, 1994). Microemulsions, which are formed at an ultra low IFT, are very difficult to demulsify since they are thermodynamically stable (Rosen, 2004). Even though a microemulsion technique provides >90% oil extraction efficiency, as shown in Table 3, it requires a high-performing demulsification process.

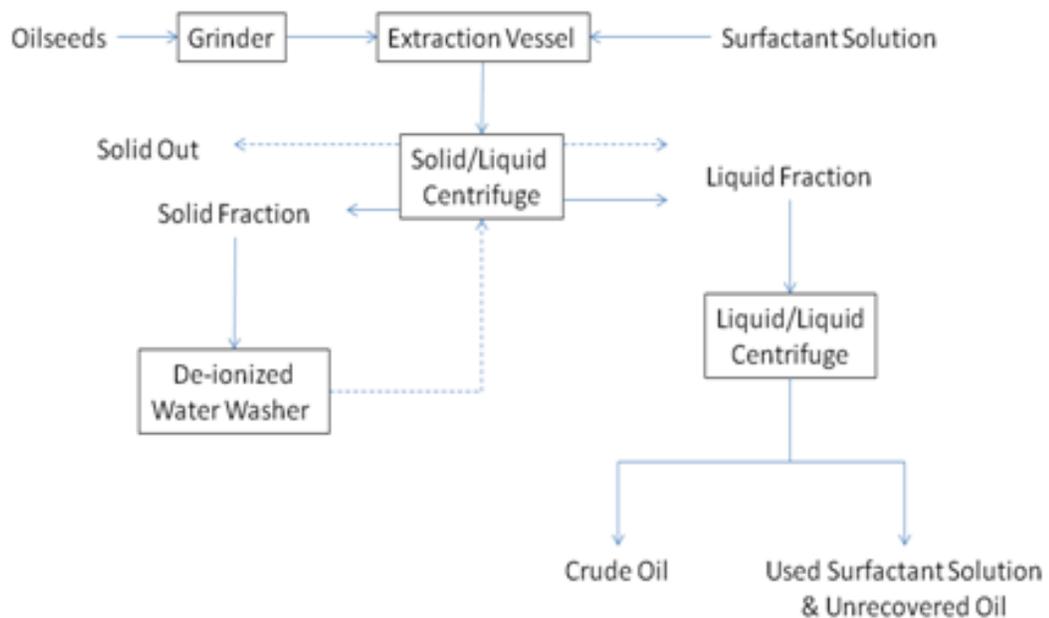


Figure 11. Schematic diagram of laboratory-based pilot scale processing of peanut and canola oil extraction. Solid line (—): surfactant wash step; Dashed line (- -): DI wash step (Do and Sabatini, 2011).



Figure 12. Selected products at different stages of ASE and DI washing at optimum conditions of peanut and canola: (a) peanut and canola flours, (b) liquid fraction from L=L centrifuge of surfactant wash step, (c) liquid fraction from L=L centrifuge of DI washing step, and (d) free crude oil recovered from L=L centrifuge (Do and Sabatini, 2011).

Table 3. Summary of the extraction process and oil extraction efficiency of aqueous surfactant-assisted extraction method.

Reference	Naksuk et al. (2009)	Do and Sabatini (2010)	Kadioglu et al. (2011)	
Oilseed and sample size	Palm kernel (1g)	Peanut and Canola (2 g)	Corn germ (4 g)	
Surfactant system	Comperland KD (3% wt)	C10-18PO-2EoSulfate (0.15 % wt)	C12,14-10PO-2EoSulfate (0.4 % wt)	
	Alfoterra 145- 5PO (0.1 % wt)	NaCl (6 % wt) for peanut	NaCl (1 % wt)	
	NaCl (10 % wt)	NaCl (5 % wt) for canola		
Pretreatment	Grinding	Food processor	Coffee mill	
	Dehulling	Yes	Not Reported	
	Size Selection	US Sieve size No.40-70 (0.21-0.42 mm)	US Sieve size No.40-70 (0.21-0.42 mm)	US Sieve size No.40-70 (0.21-0.42 mm)
	Thermal Treatment	Not Reported	104 °C with 35 mins	Yes (dried corn germ)
Extraction	Seed-Liquid ratio	1 to 10 (g/mL)	2 to 10 (g/g)	1 to 10 (g/g)
	Extraction	Not Reported	Horizontal shaker 150 cycle/min	Horizontal shaker 250 cycle/min
	Duration	with 30 min	with 30 min	with 45 min
	Separation	Not Reported	Centrifuge speed 2,170 g with 30 min	Centrifuge speed 3,500 rpm with 20 min
Oil Extraction Efficiency	93.99 % (based on soxhlet extraction)	93-95 % (based on soxhlet extraction)	83 % (based on hexane solvent in ASE)	

Table 4. Summary of quality analysis of extracted vegetable oil from Table 3.

Reference	Naksuk et.al (2009)		Do and Sabatini (2010)				Kadioglu et.al (2011)		
Oilseed	Palm Kernel		Peanut		Canola		Corn Germ		
Extraction method	Hexane	Surfactant	Hexane	Surfactant	Hexane	Surfactant	Hexane	Surfactant	Enzymatic
Color	Clear Yellow	Clear Yellow	-	-	-	-	-	-	-
Water in oil (%wt)	0.385	0.191	-	-	-	-	-	-	-
Triglycerols (%wt)	-	-	-	-	-	-	97.1	92.9	98
Free fatty acid (%wt)	-	-	0.7	0.05	0.6	0.04	-	-	-
12 h cold test	-	-	Pass	Not Pass	Pass	Not Pass	-	-	-
Triglycerol Profile									
(%wt based on total Triglycerol)									
(C18:3) : (C18:1) : (C18:1)	-	-	-	-	7.7	8.45	-	-	-
(C18:2) : (C18:2) : (C18:1)	-	-	11	14.3	-	-	-	-	-
(C18:2) : (C18:1) : (C18:1)	-	-	14.8	14	24.7	23.9	-	-	-
(C18:2) : (C18:1) : (C16:0)	-	-	10.1	11.8	-	-	-	-	-
(C18:1) : (C18:1) : (C18:1)	-	-	10	14.1	47.8	44.4	-	-	-
Fatty acid									
(%wt based on total fatty acid)									
C12:0	49.41	49.52	-	-	-	-	-	-	-
C14:0	17.56	17.44	-	-	-	-	-	-	-
C16:0	9.24	9.17	-	-	-	-	-	-	-
C18:0	2.73	2.74	-	-	-	-	-	-	-
C18:1	18.18	18.21	-	-	-	-	-	-	-
C18:2	2.88	2.92	-	-	-	-	-	-	-

### 2.3. Canola

Canola (CANadian Oil Low Acid) is the trade-marked name of a genetically modified rape seed (*Brassica napus* L.). It was developed to decrease the proportion of erucic acid, which may be toxic to humans, from 45% in regular rape seed oil to less than 2% in canola oil (Gunstone et al., 2000). The oil content of the canola seed is very economical for extraction, as the seed has an oil content of more than 40% weight. Even though there is a natural range in the fatty acid components; the main fatty acid of canola oil is oleic acid (C18:1), which accounts for more than 60% of the total fatty acids. Due to the abundance of monounsaturated fatty acid, canola oil has been applied to many products, such as cooking oil, biodiesel, lubricants, surfactants, paints, inks, and polymers (Gunstone, 2004).

Moreover, there is a growing interest in biofuel crops due to biofuel support policies. The Energy Independence & Security Act in 2007 (EISA) set the following biofuel production targets: 12.95 billion gallons in 2010, 20.5 billion gallons in 2015, and 36 billion gallons in 2022 (Cui et al., 2010). Therefore, incentives for developing biodiesel from canola oil are increasing, especially in North Dakota, which accounted for approximately 92% of US domestic canola production in 2002 (Berglund, McKay and Knodel, 2011).

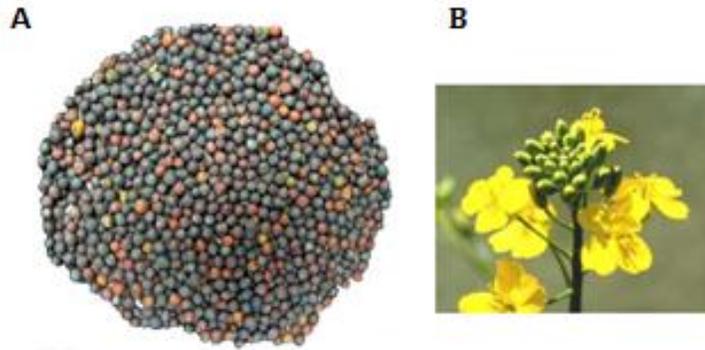


Figure 13. Pictures of (a) canola seeds and (b) a canola plant (NCCRP, 2011).

#### **2.4. Biodiesel: From Farm to Fuel Concept**

Due to the petroleum crisis in the 1990s, the price of fossil oil increased from 20 US dollars/barrel to 140 US dollars/barrel in 2004. Later, it went down to 80 US dollars/barrel in 2010 due to the economic crisis (Langpap and Wu, 2011). Many countries suffered from this situation and realized that they should have their own energy sources. However, developing fossil fuel extraction technology and/or investing in the discovery of new sources of fossil fuel cannot solve long-term energy shortage issues because fossil fuel is a nonrenewable resource. Moreover, recently, there is a growing concern about global warming. Thus, new energy sources should be renewable and environmentally friendly. One promising alternative fuel is biodiesel. Biodiesel is generated from vegetable oil and/or animal fat; the majority of which comes from vegetable oil. This makes biodiesel a renewable fuel and reduces CO<sub>2</sub> emissions to the atmosphere (Figure 14). In addition, vegetable oil, animal fat, and their derivatives are very suitable for fuel use, because they have high cetane number, which indicates the ignition quality of the fuel (Knothe, Krahl and Van Gerpen, 2004).

Due to the limitation of commercial diesel engines, vegetable oil cannot be directly used. High viscosity is the main problem of vegetable oil (Knothe et al., 2004). Therefore, vegetable oil must be transesterified to alkyl esters (biodiesel).

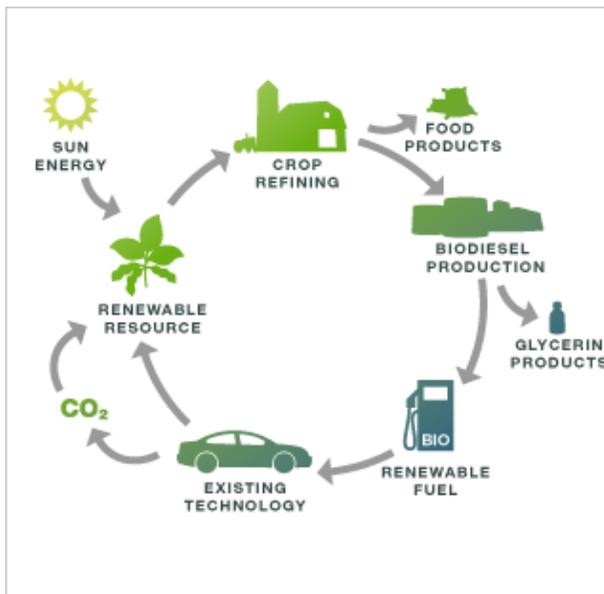


Figure 14. Biodiesel concept: from farm to fuel.

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

#### 2.4.1. Foundation of transesterification reaction

Transesterification is a reaction that breaks down the triglyceride, the main component of vegetable oil, into fatty acid alkyl ester to reduce the high viscosity of vegetable oil. The three main inputs in this reaction are the triglyceride (vegetable oil), alcohol, and a catalyst, presented in Figure 15. The two most common types of biodiesel are fatty acid methyl ester (FAME) and fatty acid ethyl ester (FAEE), because they use methanol and ethanol, which are the least expensive alkyl alcohols (Van Gerpen and Knothe, 2004).

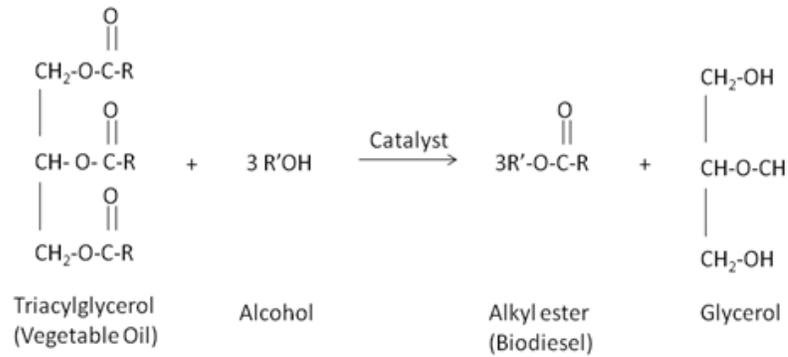


Figure 15. Transesterification reaction (Van Gerpen and Knothe, 2004).

The transesterification reaction can be classified into two main types based on the catalyst used: base and acid (Figures 16 and 17). A base catalysis, such as with sodium or potassium hydroxide, results in a much more rapid process than an acid catalysis does. However, base catalysis is not suitable for a vegetable oil that contains high amounts of free fatty acid, like used cooking oil. Therefore, acid catalysis transesterification (sulfuric acid) is frequently used as a pretreatment process prior to base catalysis transesterification in order to reduce the amount of free fatty acid by converting it into an alkyl ester.



Figure 16. Free fatty acid reacts with catalyst forming soap and water (Van Gerpen and Knothe, 2004).

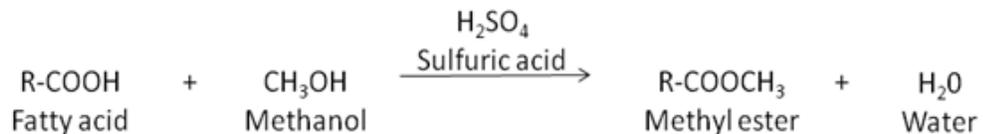


Figure 17. Free fatty acid reduction by an acid-catalyzed pretreatment (Van Gerpen and Knothe, 2004).

In order to achieve the optimum level of transesterification, several parameters were studied, such as the molar ratio of alcohol to vegetable oil, temperature, reaction time, degree of refinement of the vegetable oil, concentration of a catalyst, and effects of the presence of moisture and the free fatty acid.

#### **2.4.2. Quality of feed stock for biodiesel production**

The feed stock should be free of water; and have a free fatty acid content of less than 0.5 wt% (Freedman, Pryde and Mounts, 1984). Moreover, climate is also an important factor. In tropical climates, palm oil is predominately used in biodiesel production. However, in cold climates, palm oil biodiesel cannot be used due to the low temperatures, turning palm oil biodiesel to solid phase. Thus, rapeseed and canola oil are mainly used for biodiesel production in cold regions, because this allows engines to operate at temperatures below 0 °C (Walker, 2004). Canola biodiesel can remain liquid at low temperatures, because its main fatty acid content is unsaturated, which has a lower freezing point than palm oil (as reflected in the cloud and pour point). However, vegetable oils with high level of polyunsaturated fatty acids (as reflected in the iodine number) are very sensitive to oxidation and polymerization, creating thick sludge and clogging diesel engines. Ideally, the suitable feedstock for biodiesel should be low in saturated and polyunsaturated fatty acids (Walker, 2004).

#### **2.4.3. Biodiesel quality and standard**

In the past, biodiesel quality was evaluated using diesel No. 2 quality standards. Diesel No. 2 is used in commercial diesel engines, and the characteristics of diesel No. 2

and biodiesel are very similar. It should be noted that biodiesel is a liquid fuel that currently must pass every standard listed in ASTM D6751 as shown in Table 5.

Table 5. Biodiesel standard ASTM D6751 (American Society for Testing and Materials, 2009).

Property	Test method	Limits	Unit
Flash point (closed cup)	D 93	130.0 min	°C
Water and sediment	D 2709	0.050 max	% volume
Kinematic viscosity, 40°C	D 445	1.9-6.0	mm <sup>2</sup> /s
Sulfated ash	D 874	0.020 max	% mass
Sulfur	D 5453	0.0015 max or 0.05 max	% mass
Copper strip corrosion	D 130	No.3 max	
Cetane number	D 613	47 min	
Cloud point	D 2500	Report	°C
Carbon residue (100% sample)	D 4530	0.0350 max	% mass
Acid number	D 4664	0.80 max	mg KOH/g
Free glycerin	D 6584	0.020 max	% mass
Total glycerin	D 6584	0.240 max	% mass
Phosphorus content	D 4951	0.001 max	% mass
Distillation temperature, atmospheric equivalent temperature, 90% recovered	D 1160	360 max	°C

#### 2.4.4. Optimizing biodiesel production and quality

One problem of biodiesel production is its slow transesterification reaction rate due to the heterogeneous phases of vegetable oil and short chain alcohol with a catalyst. Some researchers have applied microwaves and/or ultrasonic waves to speed up the process. Mirgorodskaya et al. (2006), for instant, stated that using ultrasonic waves proved to be an efficient way to save time and energy in soybean transesterification. Hsiao et al. (2010) reported that they reduced the transesterification reaction time to 3 minutes by combining ultrasonic waves and microwaves in soybean biodiesel production.

The addition of a co-solvent also solves this problem. Tetrahydrofuran (THF) has been used in lots of studies, and reports state that introducing THF increased the solubilization of methanol in vegetable oil, leading to accelerated transesterification (Van Gerpen and Knothe, 2004). However, recovering and purifying methanol and co-solvent from biodiesel are more difficult. In addition, some co-solvents are hazardous chemicals.

A surfactant is an interesting co-solvent in the transesterification process. A number of papers have reported on the effects of surfactants on kinetics of transesterification process. Khan (1996), Magi et al. (2000) and Mirgorodskaya et al. (2006) stated that an applied surfactant can increase the kinetic rate of transesterification reaction. Hence the effect of the residual surfactant in the ASE extracted oil on the transesterification reaction is a very interesting subject to study.

## **2.5. References**

- American Society for Testing and Materials. 2009. "Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels." ASTM D6751-09. West Conshohocken: ASTM.
- Berglund, D. R., McKay, K. and Knodel, J. Canola Production. [Online]. 2011. Available from: <http://www.ag.ndsu.edu/pubs/plantsci/crops/a686w.htm> [21 November 2011]
- Booth, E. J. Extraction and refining. 2004. In F. D. Gunstone (eds.), Rapeseed and canola oil: production, processing, properties and uses, pp.17-36. Florida: CRC Press.

- Carlota, O., Rangel, Y., Adalberto-Jr, P. and Leoberto, C. T. 2005. Micellar solubilization of drugs. Pharmacy and Pharmaceutical Sciences 8: 147-163.
- Chabrand, R. n. M., Kim, H.-J., Zhang, C., Glatz, C. E. and Jung, S. 2008. Destabilization of the emulsion formed during aqueous extraction of soybean oil. Journal of American Oil Chemists' Society 85: 383-390.
- Cui, J., Lapan, H., Moschini, G. C. and Cooper, J. 2010. "Welfare impacts of alternative biofuel and energy policies." Agricultural & Applied Economics Association's 2011 AAEA, CAES & WAEA Joint Annual Meeting. Denver, Colorado, USA.
- David, J. 2002. Fundamentals and applications of ultrasonic waves. Florida: CRC Press.
- Davidson, M. W. Plant cell structure. [Online]. 2011. Available from: <http://micro.magnet.fsu.edu/cells/plantcell.htm> [27 November 2011]
- Do, L. D. and Sabatini, D. A. 2010. Aqueous extended-surfactant based method for vegetable oil extraction: proof of concept. Journal of American Oil Chemists' Society 87: 1211-1220.
- Do, L. D. and Sabatini, D. A. 2011. Pilot scale study of vegetable oil extraction by surfactant-assisted aqueous extraction process. Separation Science and Technology 46(6): 978-985.
- Freedman, B., Pryde, E. H. and Mounts, T. L. 1984. Variables affecting the yields of fatty esters from transesterified vegetable oils. Journal of American Oil Chemists' Society 61(10): 1638-1643.
- Gregory, S. and Horsman, M. Who needs alternative solvents, and criteria for their selection 1997. In P. J. Wan and P. J. Wakelyn (eds.), Technology and solvents for extraction oilseeds and nonpetroleum oils, pp.1-3. Champaign: AOCS Press.

- Gunstone, F. D. 2004. Rapeseed and canola oil: production, processing, properties and uses. Florida: CRS Press.
- Gunstone, F. D., Hamilton, R. J., Cast, J., Jee, M. and Hamm, W. 2000. Vegetable oils in food technology: composition, properties and uses. Florida: CRC Press.
- Hsiao, M. C., Lin, C. C., Chang, Y. H. and Chen, L. C. 2010. Ultrasonic mixing and closed microwave irradiation-assisted transesterification of soybean oil. Fuel 89: 3618-3622.
- Johnson, L. A. Theoretical, comparative, and historical analyses of alternative technologies for oilseeds extraction. 1997. In P. J. Wan and P. J. Wakelyn (eds.), Technology and solvents for extraction oilseeds and nonpetroleum oils, pp.4-47. Champaign: AOCS Press.
- Kadioglu, S. I., Phan, T. T. and Sabatini, D. A. 2011. Surfactant-based oil extraction of corn germ. Journal of American Oil Chemists' Society 88: 863-869.
- Khan, L. M. and Hanna, M. A. 1983. Expression of oil from oilseeds: a review. Journal of Agricultural and Engineering Research 28: 495-503.
- Khan, M. N. 1996. Effects of mixed anionic and cationic surfactants on rate of transesterification and hydrolysis of esters. Journal of Colloid and Interface Science 182: 602-605.
- Klongklaew, N., Sabatini, D. A. and Tongcumpou, C. 2005. Hexane replacement for soybean extraction using microemulsion systems. Master's Thesis, Program in Environmental Management interdisciplinary Graduate School, Chulalongkorn University.

- Knothe, G., Krahl, J. and Van Gerpen, J. 2004. The biodiesel handbook. Champaign: AOCS Press.
- Kunjappu, J. T. Ink chemistry. [Online]. 2003. Available from: <http://www.rsc.org/chemistryworld/issues/2003/march/inkchemistry.asp>. [16 April 2011]
- Langpap, C. and Wu, J. 2011. Potential environmental impact of increasing reliance on corn-based bioenergy. Environmental Resource Economics 49: 147-171.
- Li, H., Song, C., Zhou, H., Wang, N. and Cao, D. 2011. Optimization of the aqueous enzymatic extraction of wheat germ oil using response surface methodology. Journal of American Oil Chemists' Society 88: 809-817.
- Magi, K., Nakajima, M. and Mukataka, S. 2000. Transesterification reaction between medium and long chain fatty acid triglycerides using surfactant-modified lipase. Biotechnology and Bioengineering 67(5): 513-519.
- Mirgorodskaya, A. B., Kudryavtseva, L. A., Vylegzhanina, N. N., Zuev, Y. F. and Idiyatullin, B. Z. 2006. Catalytic properties of microheterogeneous systems on cationic surfactants in transesterification processes. Kinetics and Catalysis 47(1): 5-11.
- Naksuk, A., Sabatini, D. A. and Tongcumpou, C. 2009. Microemulsion-based palm kernel oil extraction using mixed surfactant solutions. Industrial Crops and Products 30: 194-198.
- NCCRP. Canola picture. [Online]. 2011. Available from: <http://www.ag.ndsu.edu/nc-canola/> [13 June 2011]

- Rosen, M. J. 2004. Surfactant and Interfacial Phenomena. 2nd ed. New York: John Wiley & Sons.
- Rosenthal, A., Pyle, D. L. and Niranjana, K. 1996. Aqueous and enzymatic process for edible oil extraction. Enzyme and Microbial Technology 19: 402-420.
- Sabatini, D. A., Acosta, E. and Harwell, J. H. 2003. Linker molecules in surfactant mixtures. Current Opinion in Colloid and Interface Science 8: 316-326.
- Shah, S., Shana, A. and Gupta, M. N. 2004. Extraction of oil from *Jatropha curcas* L. seed kernels by combination of ultrasonication and aqueous enzymatic oil extraction. Biosource Technology 96: 121-123.
- Swanson, B. G. Hexane extraction in soyfood processing. [Online]. 2012. Available from:<http://www.soyfoods.org/wp-content/uploads/Regulatory%20Expert%20Document-Barry%20Swanson%20revised.pdf> [20 June 2012]
- Tongcumpou, C., Acosta, E. J., Scamehorn, J. F., Sabatini, D. A., Yanumet, N. and Chavadej, S. 2006. Enhanced triolein removal using microemulsions formulated with mixed surfactants. Journal of Surfactants and Detergents 9(2): 181-189.
- U.S. Environmental Protection Agency (EPA). 2001. "National emission standards for hazardous air pollutants." Solvent extraction for vegetable oil production. Washington, DC: EPA.
- Uchiyama, H., Acosta, E., Tran, S., Sabatini, D. A. and Harwell, J. H. 2000. Supersolubilization in chlorinated hydrocarbon microemulsions: solubilization enhancement by lipophilic and hydrophilic linkers. Industrial Engineering Chemistry Research 39: 2704-2708.

- Van Gerpen, J. and Knothe, G. Basic of the transesterification reaction. 2004. In G. Knothe, J. Krahl and J. Van Gerpen (eds.), The biodiesel handbook, pp.26-41. Champaign: AOCS Press.
- Walker, K. Non-food uses. 2004. In F. D. Gunstone (eds.), Rapeseed and canola oil: production, processing, properties and uses, Florida: CRC Press.
- Wang, S. S., Lee, C. J. and Chan, C. C. 1994. Demulsification of water-in-oil emulsion by use of a high voltage ac field. Separation Science and Technology 29(2): 159-170.
- Wisnborn, D., Glowacki, A., Hettiarachchy, N. and Zander, L. 1993. A batch-scale unit for continuous extraction of red colorant from sunflower hulls. Transaction of the ASAE 36(5): 1441-1416.
- Witthayapanyanon, A., Acosta, E. J., Harwell, J. H. and Sabatini, D. A. 2006. Formulation of ultralow interfacial tension systems using extended surfactants. Journal of Surfactants and Detergents 9: 331-339.

**CHAPTER 3. PAPER 1: IMPROVING CANOLA OIL DETACHMENT  
EFFICIENCY BY REDUCING INTERFACIAL TENSION**

Paper 1 is an edited and revised version of a paper presented at the ASABE/CSBE North Central Intersectional Conference in Fargo, ND, March 30-31, 2012. Authors: Nattapong Tuntiwiwattanapun, Dennis Wiesenborn, and Chantra Tongcumpou. Title: Optimization of Vegetable Oil Detachment Efficiency by Reducing Interfacial Tension. Paper number: 12113. Nattapong Tuntiwiwattanapun, the author of this thesis, is the first author of Paper 1. He designed and conducted the experiment in this paper and also wrote Paper 1, which was edited by the other co-authors.

### **3.1. Abstract**

Due to the growing concern of using hexane in vegetable oil extraction, an aqueous surfactant-assisted extraction (ASE) was evaluated for potential use as an alternative to hexane extraction. Among three commercial anionic surfactants in this study, sodium dodecyl sulfate (SDS) was the most suitable surfactant for canola oil, since it provided lower dynamic interfacial tension (IFT) than the other two. Mixtures of SDS and nonionic surfactant solution generated lower dynamic IFT than SDS solution alone. Introducing NaCl further reduced dynamic IFT but only within the specific concentration range. At higher concentrations of NaCl, surfactant precipitation was observed. However, There was no significant difference in oil detachment efficiency between the conditions that provided the lowest dynamic IFT (0.148 mN/m) and the highest dynamic IFT (0.869 mN/m) in this study. The highest oil detachment efficiency of canola was 80.7%, with a dynamic IFT value of 0.869 mN/m using 0.03 M SDS without NaCl as an extraction medium.

### **3.2. Introduction**

Vegetable oil is typically extracted via hexane solvent extraction due to low operation cost and high oil extraction efficiency. However, there are growing concerns over safety, health and environmental quality for that process. Hexane is a volatile organic compound (VOC) that has been categorized as a hazardous air pollutant and issued a strict emission limit of 100 mg/Nm<sup>3</sup> by the U.S. Environmental Protection Agency (EPA) (2001). For one ton of oilseed processed there was between 0.7-4.5 L of hexane lost in the oil extraction process (U.S. Environmental Protection Agency (EPA),

2001). Workers who have been exposed to hexane at 15 ppm/day for 3 months showed peripheral nerve damage (Do and Sabatini, 2010). Moreover, hexane is typically found in refined vegetable oil at a level of 0.8 mg/kg (Swanson, 2012). Thus, oilseed processors are required to fully contain hexane, have a leak detection system, and good solvent recycling processes. These concerns warrant the exploration of a hexane-free vegetable oil extraction method.

Several solvents have been evaluated to replace hexane in a vegetable oil extraction process such as: water (Hanmoungjai, Pyle and Niranjan, 2000), ethanol (Kwiatkowski and Cheryan, 2002), acetone (Youngs and Sallans, 1955), heptanes and isohexane (Wan et al., 1995), isohexane with alcohol mixture (Kuk and Hron, 1998), mixed propane with supercritical fluid CO<sub>2</sub> (Pederssetti et al., 2011) and even vegetable oil itself (Strop and Perry, 1989). Recently, aqueous extraction processes have been developed by introducing enzyme or surfactant to water for improving the oil extraction efficiency (Do and Sabatini, 2010; Dominguez, Ndfiez and Lema, 1994; Ghosh, Jayas and Agrawal, 2007; Kadioglu, Phan and Sabatini, 2011; Latif, Diosady and Anwar, 2008; Naksuk, Sabatini and Tongcumpou, 2009)

Surfactants (surface active agent) are amphipathic substances in which the molecule contains both hydrophilic (polar) and hydrophobic (non polar) parts. Typically, polar (water) and non polar (vegetable oil) compounds cannot be homogeneously mixed due to their high interfacial tension (IFT). By definition, IFT is the repulsive energy between two different species of molecules (Rosen, 2004). It is the minimum energy (heat, gravity and shear force) that is required to make one phase disperse into another

phase, thereby increasing the surface area between two different phases. Due to their unique structure, surfactants can reduce IFT and hence help oils disperse into the bulk water phase. It has been used in many applications, including cleaning, foods, paint, cosmetics, drug delivery, material synthesis and site remediation (Rosen, 2004). In this work, surfactants were applied to improve the aqueous extraction method, named aqueous surfactant-assisted extraction (ASE).

Water alone cannot extract the trapped oil inside an insoluble matrix of denatured proteins (Campbell and Glatz, 2009). High temperature and shear force must be applied to extract this trapped oil. A reduction in IFT results in a reduction in the size of the oil droplet. Smaller oil droplets more readily diffuse from the disrupted cell matrix, and then disperse into extraction medium (Do and Sabatini, 2010). A reduction in IFT also promotes the oil detachment from oilseed surface to extraction media. Therefore, selecting a suitable surfactant system, which provides the low IFT value, is one of the most important steps for the ASE method. The hydrophilic-lipophilic balance (HLB) value is commonly used to choose a suitable surfactant. It indicates the ratio between polar and non-polar parts of the surfactant molecular structure. Surfactants with an HLB value similar to that of the oil provide a low IFT value. The HLB value of vegetable oil is very difficult to accurately determine, since its structure is very complex consisting of many different species of molecules. However, the HLB value is still worth using as a guideline for selecting a suitable surfactant system for the ASE method. Co-solvent, electrolyte, temperature, and various surfactants are widely applied to modify the HLB value (Naksuk et al., 2009; Sabatini, Acosta and Harwell, 2003; Salager et al., 1979; Tongcumpou et al., 2006).

Several reports show that the IFT should be reduced to 0.01 mN/m (ultralow level) by incorporation of surfactant with co-solvent or use of extended surfactant (Do and Sabatini, 2010; Do and Sabatini, 2011; Kadioglu et al., 2011; Naksuk et al., 2009). However, use of volatile co-solvent, such as short chain alcohols, requires a leak-proof system. Extended surfactants are not yet commercially available. Hence, the main objective of this study was to select a suitable commercial surfactant system without co-solvent, which provides the desired low IFT value with canola oil, and then demonstrate the potential of that surfactant system as an extraction medium for canola oil.

The primary objective of this work was to select a suitable commercial anionic surfactant and improve the dynamic IFT for ASE canola oil extraction. Three commercial anionic surfactants were investigated for their effect on dynamic IFT with canola oil. The effect of introducing nonionic surfactant and electrolyte concentration on improving the dynamic IFT value of selected commercial anionic surfactant and canola oil were determined. Finally, the correlation between IFT and oil detachment efficiency was evaluated.

### **3.3. Materials and Methods**

#### **3.3.1. Materials**

Sodium bis(ethylhexyl) sulfosuccinate (AOT) and sodium dihexyl sulfosuccinate (AMA) were from Fisher Scientific Company LLC (Atlanta, GA). Sodium dodecyl sulfate (SDS) and hexane were from Sigma-Aldrich Co. LLC (St Louis, MO). Four nonionic surfactants, the fatty alcohol ethylene oxide series (LS-series), were kindly received from PTT Chemical Public Company Limited (Thailand). The molecular

structure of each surfactant is shown in Appendix A (Table 9). NaOH with 99% purity was from Fluka Chemical Corp. (Milwaukee, WI). Wesson® pure canola oil was from ConAgra Food, Inc. (Omaha, NE). Canola seeds were kindly provided by Dr. Mukhles Rahman of the Plant Sciences Department of North Dakota State University.

### 3.3.2. Dynamic interfacial tension measurement

The dynamic IFT between surfactant system solution and canola oil was measured by a spinning drop tensiometer (model SVT20), purchased from Dataphysic® (Filderstadt, Germany). The analysis procedure was modified from Witthayapanyanon et al. (2006). The surfactant solution served as the dense phase, while canola oil was used as the light phase at 25°C with spinning speed at 6,000 rpm. In this work, dynamic IFT was defined as the IFT between the fresh surfactant system solution and the canola oil after 30 min, at which time the IFT value became stable.

### 3.3.3. Hydrophile-lipophile balance value calculation

The hydrophile-lipophile balance (HLB) value is the number between 1 and 40 which indicates the emulsification behavior. HLB value also relates to the balance between the hydrophilic and lipophilic portions of the surfactant molecule (Rosen, 2004). In the case of mixed surfactants, calculation of HLB is based on weight fraction of each surfactant (Equation 2).

$$HLB \text{ of mixture surfactant} = (HLB_A \times X_A) + (HLB_B \times (1 - X_A)) \quad (\text{Equation 2})$$

where  $HLB_A$  and  $HLB_B$  are HLB value of surfactant “A” and “B”, respectively.  $X_A$  is weight fraction of surfactant “A” in the system (Rosen, 2004).

#### **3.3.4. Oilseed pretreatment**

Prior to pretreatment, canola seeds were evaluated for moisture content by a grain moisture analyzer (GAC 2100, DICKEY-john®, Auburn, IL). The moisture content was approximately 5 wt% (wet basis). Canola seeds were ground for 30 s by a coffee grinder (Protor-Silex model E160BY, Hamilton Beach Brands, Inc., Southern Pines, NC). Ground canola seed particles were then fractionated by sieve size using RO-TAP testing sieve shaker (The W.S. Tyler Company, Cleveland, OH). Only the fraction between 0.212 and 0.425 mm was used in this study, as recommended for oilseed extraction (Do and Sabatini, 2010; Kadioglu et al., 2011; Naksuk et al., 2009).

#### **3.3.5. Aqueous surfactant-assisted extraction (ASE)**

The ASE procedure is diagrammed in Figure 18. Ground canola (4 g) was suspended in 40 mL of surfactant solution. The suspension was then mixed for 30 min at 1,000 rpm on a digital hot plate/stirrer (PMC Data Plate® 730, Barnstead, Intl., Dubuque, IA). The slurry was immediately centrifuged in a bench-top-centrifuge for 20 min at 2,000 X g (Model CR412, Jouan, Inc., Winchester, VA). After centrifugation two fractions were obtained: liquid fraction (oil, emulsion, and extraction medium) and extracted meal. The extracted meal was dried in a hot air oven at 105°C over night for further oil detachment efficiency evaluation.

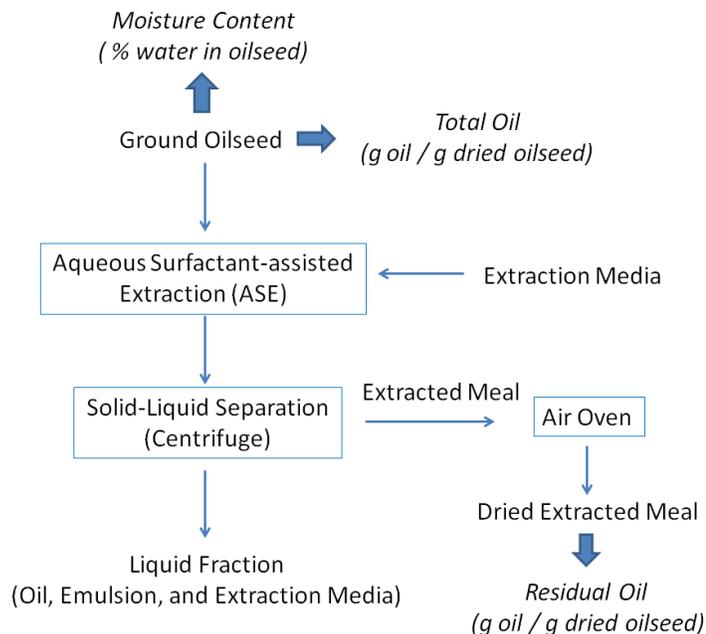


Figure 18. Diagram of aqueous surfactant-assisted extraction in Paper 1.

### 3.3.6. Oil content of oilseed and extracted meal

Oil contents of ground oilseed and of extracted canola meal were determined via a Soxhlet extractor, using hexane as solvent, as described in the AOCS official methods AM2-93 (American Oil Chemists' Society, 1999). The two oil contents were defined as the total oil (TO) and residual oil (RO) (g oil / g dried oilseed or meal), respectively. The oil detachment efficiency was then calculated as shown in Equation 3.

$$\text{Oil Detachment Efficiency (\%)} = \frac{(TO-RO)}{TO} \times 100\% \quad (\text{Equation 3})$$

### 3.3.7. Experimental design

**3.3.7.1. Anionic surfactant selection.** Three commercial anionic surfactant solutions (1 wt%): AOT, AMA and SDS were evaluated for their dynamic IFT with

canola oil. The surfactant solution that provided the lowest dynamic IFT was selected for the subsequent experiment.

**3.3.7.2. Effect of additional nonionic surfactant.** The impact of additional nonionic surfactant (LS-series) on dynamic IFT between anionic surfactant solution and canola oil was investigated. Four nonionic surfactants: LS-1, 2, 3 and 9 (a fatty alcohol ethylene oxide series) were introduced to the anionic surfactant solution selected from the previous experiment. The final surfactant solution was comprised of 1 wt% anionic surfactant and 0.01 M nonionic surfactant.

**3.3.7.3. Effect of electrolyte.** The impact of electrolyte (NaCl) concentration on dynamic IFT between the surfactant system from experiment 3.3.7.2 and canola oil was investigated. The NaCl concentration was varied between 0 and 0.5 M in increments of 0.025 M.

**3.3.7.4. Effect of interfacial tension on ASE.** The surfactant systems determined in experiment 3.3.7.3 with various NaCl concentrations were utilized as extraction medium in the ASE method as described above. The resulting oil detachment efficiency was then correlated to dynamic IFT.

### **3.3.8. Statistical analysis**

All extraction experiments were performed in triplicate. Error bars on charts were representative of  $\pm 1$  SD. Data were analyzed using the general linear models (GLM) procedure (SAS Institute, 2008). An F-protected LSD ( $P \leq 0.05$ ) was calculated for comparisons of main effect means. Significant differences ( $P \leq 0.05$ ) between means of

two way and higher order interactions were determined as twice the standard error of the mean (Snedecor and Cochran, 1980).

### 3.4. Results and Discussion

#### 3.4.1. Anionic surfactant selection

Among three anionic surfactants in this study, SDS provided the lower dynamic IFT with canola oil (Figure 19). One explanation of this result was the suitable molecular structure between surfactant and canola oil. Comprised of the same sulfosuccinate group, AOT gained the lower IFT than AMA. This might be due to the longer carbon chain length of AOT, which was more compatible with canola oil. However, with the shortest carbon chain length, SDS provided the lowest dynamic IFT. The sulfate group of SDS might compensate for its shorter carbon chain length.

Changes in carbon chain length and functional groups of surfactants impact the Winsor R ratio (R) of the oil-surfactant-water system. This ratio reflects the proportion between net interaction energy of hydrophobic and hydrophilic parts of the system, consisting of vegetable oil (O), water (W) and surfactant (S) as presented in Equation 4. The lowest IFT is produced when R=1, and the value of Net  $A_{SO}$  and Net  $A_{SW}$  are high (Rosen, 2004). As a result, SDS was selected for subsequent experiments.

$$R = \frac{Net A_{SO}}{Net A_{SW}} = \frac{A_{SO} - A_{OO} - A_{LL}}{A_{SW} - A_{WW} - A_{HH}} \quad (\text{Equation 4})$$

where Net  $A_{SO}$  is the net interaction between the surfactant and oil (hydrophobic), Net  $A_{SW}$  is the net interaction between the surfactant and water (hydrophilic),  $A_{SO}$  is the interaction between the surfactant and oil,  $A_{SW}$  is the interaction between the surfactant

and water,  $A_{OO}$  is the interaction energy among oil molecules,  $A_{LL}$  is the interaction among the tails of the surfactant molecules,  $A_{WW}$  is the interaction energy among the water molecules, and  $A_{HH}$  is the interaction among the surfactant heads (Witthayapanyanon et al., 2006).

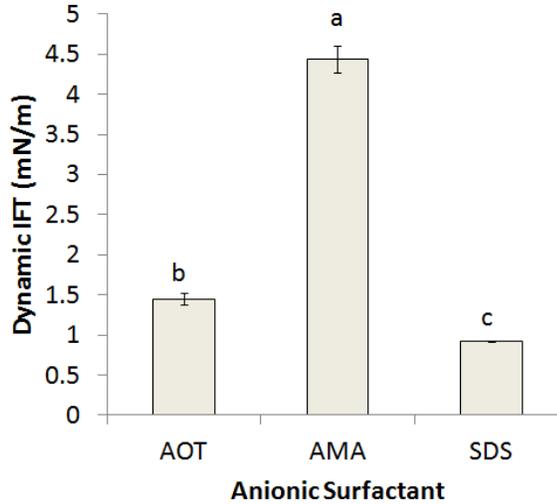


Figure 19. Dynamic IFT between 1 wt% anionic surfactant solution (AOT, AMA and SDS) and canola oil. Means within the bar graph followed by the same letter are not significantly different at  $P \leq 0.05$ .

### 3.4.2. Effect of additional nonionic surfactant

Introducing nonionic surfactant LS-series into 0.03 M SDS solution reduced the dynamic IFT of the SDS solutions with canola oil, except the mixed surfactant solution of LS-9 (Figure 20). Similar results with mixed surfactant solution causing a decrease IFT (positive synergism effect) and increase IFT (negative synergism effect) are observed in several studies (Naksuk et al., 2009; Tongcumpou et al., 2006; Zhu et al., 2009). The change in these dynamic IFT values can be explained via modification of the HLB value

of the surfactant system due to the presence of nonionic surfactant, and hence the effect on the Winsor R ratio (Table 6). The Winsor R ratio of anionic surfactant is naturally  $< 1$  due to its high water solubilization (high Net  $A_{SW}$ ). Introducing a nonionic surfactant (low HLB value surfactant) reduces the HLB value of the surfactant system, resulting in increased Net  $A_{SO}$ . Therefore, the Winsor R ratio was closer to 1. However, further decreasing the HLB value tends to increase the Winsor R ratio ( $R > 1$ ), and hence increases the IFT value. The “U” shape correlation between HLB value and IFT was observed (Figure 20). However, dynamic IFT values of these systems were still higher than the suggested level of  $< 0.01$  mN/m (Do and Sabatini, 2010; Naksuk et al., 2009). Therefore, all surfactant systems were selected for the next experiment, except the mixed surfactant solution of LS-9.

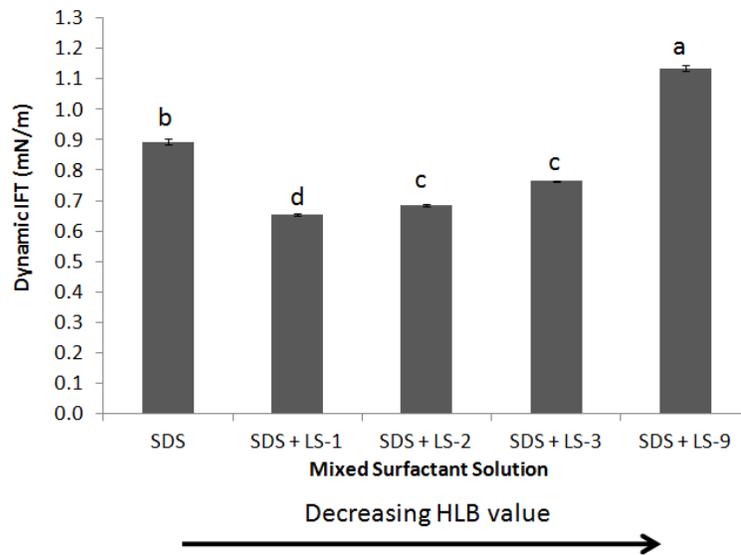


Figure 20. Dynamic IFT of canola oil with mixed solution comprised of 0.03 M SDS and 0.01 M LS-series. Means within the bar graph followed by the same letter are not significantly different at  $P \leq 0.05$ .

### 3.4.3. Effect of electrolyte

Increasing electrolyte (NaCl) concentrations affected the dynamic IFT value of every surfactant system from experiment 2. The electrolyte concentration providing the lowest IFT in the surfactant system is named optimum salinity ( $S^*$ ) (Rosen, 2004). Increasing the NaCl concentration to  $S^*$  results in reduction of dynamic IFT. However, further increasing NaCl concentration tends to increase the dynamic IFT value (Figure 21). Salt or electrolyte is commonly used in ionic surfactant solutions to modify the IFT value by changing the Winsor R ratio (Do and Sabatini, 2010; Kadioglu et al., 2011; Naksuk et al., 2009; Salager et al., 1979; Tongcumpou et al., 2006; Witthayapanyanon et al., 2006; Zhu et al., 2009). The electrolyte molecules are inserted between surfactant monomers in micelle molecules, leading to a decrease in electrical interaction between head groups of ionic surfactants. The  $A_{SW}$  and Net  $A_{SW}$  are reduced, thus an increase in Winsor R ratio, as shown in Eq. 3 (Rosen, 2004; Witthayapanyanon et al., 2006). At  $S^*$ , the Winsor R ratio is equal to 1 resulting in the lowest IFT. After  $S^*$ , the Winsor R ratio is  $>1$ , thus IFT was increased. However, only 0.03 M SDS solution was selected for the next experiment, since the surfactant precipitation was observed in the mixed surfactant system at high NaCl concentration (Figure 22).

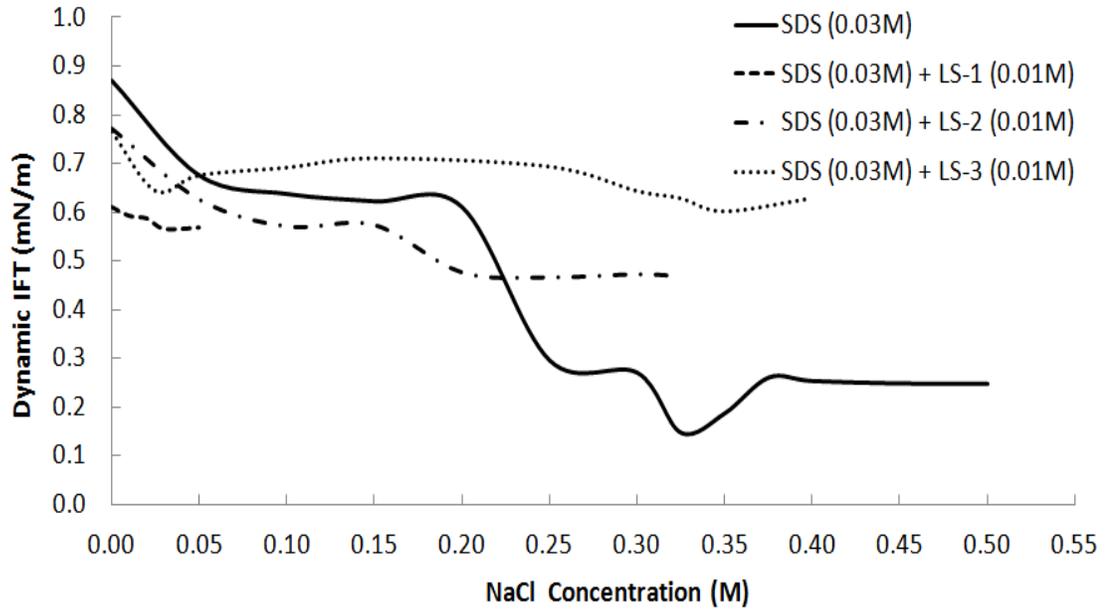


Figure 21. Effect of NaCl concentration on dynamic IFT of each surfactant system with canola oil.

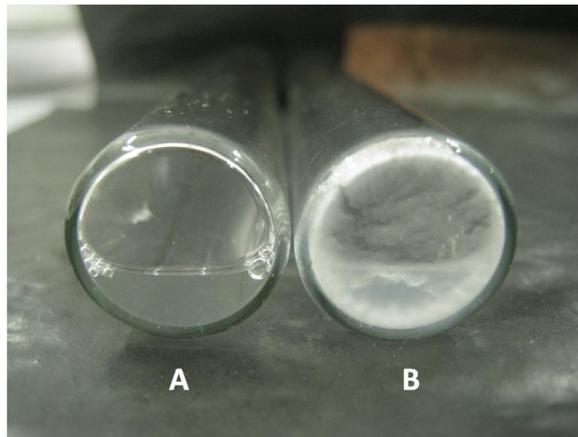


Figure 22. Surfactant of 0.2 M NaCl with (a) 0.03 M SDS and (b) 0.03 M SDS plus 0.01 M LS-1.

Table 6. HLB value and the dynamic IFT at optimum salinity of each surfactant system.

Surfactant System	HLB	Mixed HLB	S* (M)	Dynamic IFT (mN/m)
SDS (0.03 M)	40	40	0.325	0.148±0.001
SDS (0.03 M)	40			
LS-1 (0.01 M)	3.6	31.99	0.03	0.565±0.004
SDS (0.03 M)	40			
LS-2 (0.01 M)	6.1	31.53	0.1	0.571±0.006
SDS (0.03 M)	40			
LS-3 (0.01 M)	7.9	31.1	0.35	0.602±0.008
SDS (0.03 M)	40			
LS-9 (0.01 M)	13.4	29.15	0	1.136±0.010

#### 3.4.4. Effect of interfacial tension on ASE

The correlation between dynamic IFT and oil detachment efficiency was not significant ( $P \leq 0.05$ ) in this study (Figure 23). The low dynamic IFT value implied that the energy requirement (shear force, heat, and gravity) for dispersing vegetable oil to extraction medium was low. Thus, at the otherwise extraction conditions (temperature, stirring speed, and extraction time) reducing IFT increases the oil detachment efficiency. This correlation has been observed in many works by using the ultralow IFT (0.01 mN/m) with a horizontal shaker at 150-250 cycle/min (Do and Sabatini, 2010; Kadioglu et al., 2011; Naksuk et al., 2009). Even though the lowest dynamic IFT value in this study was 10 times higher than the suggested level, an encouraging level of oil detachment efficiency was achieved. The oil detachment efficiency at the highest dynamic IFT, 0.869 mN/m, was not significantly different from that of the lowest dynamic IFT, 0.148 mN/m.

This might be due to the high shear force from the magnetic stirrer in this study compensating for the high dynamic IFT.

Moreover, the commercial canola oil used in IFT measurement was highly refined. Undesirable components in crude oil, such as chlorophyll, phospholipids and proteins, were removed. Phospholipids and proteins are amphiphilic substances, thus their presence affects the IFT of the system. Therefore, a dynamic IFT value from commercial canola oil was not representative of crude oil that was extracted by the ASE method. Crude oil from a screw press extraction should be a better sample for IFT measurement. As a result, the highest oil detachment efficiency of canola was 80.7%, with a dynamic IFT value of 0.869 mN/m (0.03 M SDS without NaCl).

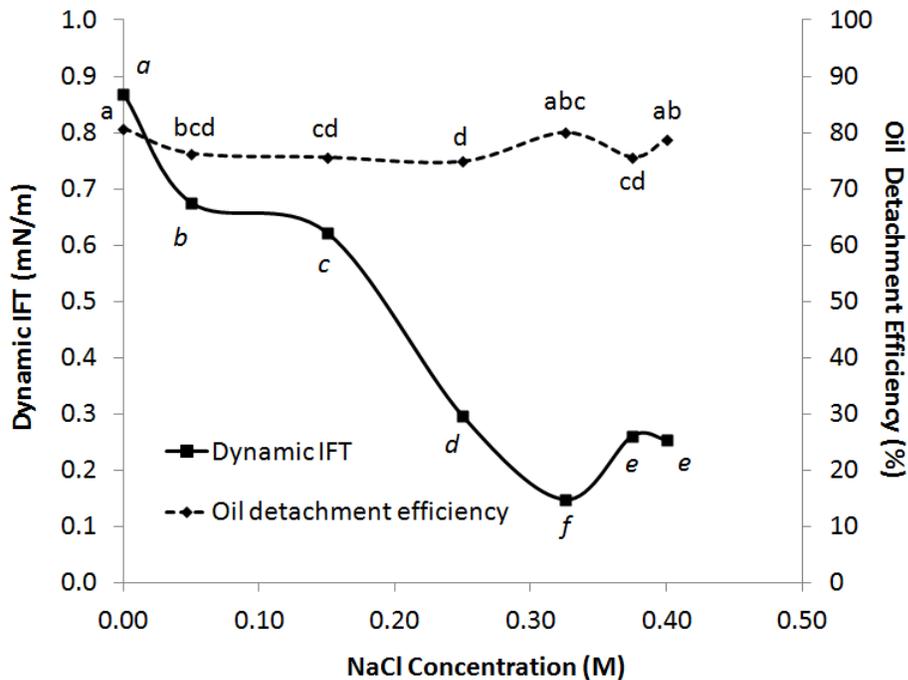


Figure 23. Scatter plot of dynamic IFT and oil detachment efficiency at various NaCl concentrations of canola oil with 0.03M SDS solution. Means within the line graph followed by the same letter are not significantly different at  $P \leq 0.05$ .

### **3.5. Conclusions**

The commercial anionic surfactant SDS solution showed good potential for use as an extraction medium in the ASE method. The addition of the 0.01 M nonionic surfactant LS-series and introduction of NaCl were found to improve the dynamic IFT of the system. However, surfactant precipitation was a limitation to introducing NaCl into the system. Although an ultralow IFT ( $<0.01$  mN/m) was not observed in this study, more than 70% of the oil detachment efficiency was achieved. Unlike the other studies on the ASE method, a correlation between dynamic IFT and oil detachment efficiency was not observed. There was no significant difference in oil detachment efficiency between the conditions that provided the lowest dynamic IFT (0.148 mN/m) and the highest dynamic IFT (0.869 mN/m) in this study.

These results suggest that dynamic IFT of the system plays an important role in oil detachment efficiency. Other parameters such as mixing conditions may also have an effect on the extraction performance. However, using high shear force to compensate for the dynamic IFT  $> 0.01$  mN/m promoted emulsion formation, and a demulsification process was required. The highest oil detachment efficiency of canola was 80.7% with a dynamic IFT value 0.869 mN/m (0.03 M SDS). Lastly, it should be emphasized that the ASE method offered several advantages over the hexane extraction, particularly the absence of toxic compounds and the ability to operate at room temperature.

### **3.6. Acknowledgements**

The authors would like to thank the Higher Education Research Promotion and National Research University Project of Thailand, the Office of the Higher Education

Commission, the Center of Excellence for Environmental and Hazardous Waste Management (Thailand) and North Dakota Agricultural Experiment Station, and the North Dakota Center of Excellence for Oilseeds Development for providing the experimental facilities and financial support; Darrin Haagensohn of the Agricultural & Biosystems Engineering Dept., North Dakota State University (NDSU) for technical support; Dr. Mukhlesur Rahman of Plant Science Dept., NDSU for providing canola seed; and Dr. Eakalak Khan of Civil Engineer Dept., NDSU for providing the great opportunity to Nattapong Tuntiwiwattanapun for studying at NDSU.

### **3.7. References**

- American Oil Chemists' Society. 1999. "Oil content in oilseeds." AOCS method AM2-93. Champaign, IL: AOCS.
- Campbell, K. A. and Glatz, C. E. 2009. Mechanisms of aqueous extraction of soybean oil. Agricultural and Food Chemistry 57: 10904-10912.
- Do, L. D. and Sabatini, D. A. 2010. Aqueous extended-surfactant based method for vegetable oil extraction: proof of concept. Journal of American Oil Chemists' Society 87: 1211-1220.
- Do, L. D. and Sabatini, D. A. 2011. Pilot scale study of vegetable oil extraction by surfactant-assisted aqueous extraction process. Separation Science and Technology 46(6): 978-985.
- Dominguez, H., Ndzifon, M. J. and Lema, J. M. 1994. Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: a review. Journal of Food Chemistry 49: 271-286.

- Ghosh, P. K., Jayas, D. S. and Agrawal, Y. C. 2007. "Enzymatic hydrolysis of oilseeds for enhanced oil extraction: current status." Trans. ASABE. ASABE Annual International Meeting. Minneapolis, Minnesota: ASABE. Vol. 076207.
- Hanmoungjai, P., Pyle, L. and Niranjan, K. 2000. Extraction of rice bran oil using aqueous media. Journal of Chemical Technology and Biotechnology 75: 348-352.
- Kadioglu, S. I., Phan, T. T. and Sabatini, D. A. 2011. Surfactant-based oil extraction of corn germ. Journal of American Oil Chemists' Society 88: 863-869.
- Kuk, M. S. and Hron, R. J. 1998. Cottonseed extraction with a new solvent system: isohexane and alcohol mixtures. Journal of American Oil Chemists' Society 75: 927-930.
- Kwiatkowski, J. R. and Cheryan, M. 2002. Extraction of oil from ground corn using ethanol. Journal of American Oil Chemists' Society 79: 825-830.
- Latif, S., Diosady, L. L. and Anwar, F. 2008. Enzyme-assisted aqueous extraction of oil and protein from canola (*Brassica napus* L.) seeds. European Journal of Lipid Science and Technology 110: 887-892.
- Naksuk, A., Sabatini, D. A. and Tongcumpou, C. 2009. Microemulsion-based palm kernel oil extraction using mixed surfactant solutions. Industrial Crops and Products 30: 194-198.
- Pederssetti, M. M., Palú, F., Silva, E. A. d., Rohling, J. H., Cardozo-Filho, L. and Dariva, C. 2011. Extraction of canola seed (*Brassica napus*) oil using compressed propane and supercritical carbon dioxide. Journal of Food Engineering 102: 189-196.

- Rosen, M. J. 2004. Surfactant and Interfacial Phenomena. 2nd ed. New York: John Wiley & Sons.
- Sabatini, D. A., Acosta, E. and Harwell, J. H. 2003. Linker molecules in surfactant mixtures. Current Opinion in Colloid and Interface Science 8: 316-326.
- Salager, J. L., Morgan, J. C., Schechter, R. S., Wade, W. H., Spe-Aime, M. and E.Vasquez. 1979. Optimum formulation of surfactant/water/oil systems for minimum interfacial tension or phase behavior. Society of Petroleum Engineers 19: 107-115.
- SAS Institute. 2008. "Release version 9.2 for window." Cary: SAS Institute.
- Snedecor, G. W. and Cochran, W. G. 1980. Statistical Methods. 7th ed. Ames: Iowa State University Press.
- Strop, H. R. and Perry, R. R. 1989. "Vegetable oil extraction process." USA. Vol. 4,808,426.
- Swanson, B. G. Hexane extraction in soyfood processing. [Online]. 2012. Available from:<http://www.soyfoods.org/wp-content/uploads/Regulatory%20Expert%20Document-Barry%20Swanson%20revised.pdf> [20 June 2012]
- Tongcumpou, C., Acosta, E. J., Scamehorn, J. F., Sabatini, D. A., Yanumet, N. and Chavadej, S. 2006. Enhanced triolein removal using microemulsions formulated with mixed surfactants. Journal of Surfactants and Detergents 9(2): 181-189.
- U.S. Environmental Protection Agency (EPA). 2001. "National emission standards for hazardous air pollutants." Solvent extraction for vegetable oil production. Washington, DC: EPA.

- Wan, P. J., Hron, R. J., Dowd, M. K., Kuk, M. S. and Conkerton, E. J. 1995. Alternative hydrocarbon solvents for cottonseed extraction: plant trials. Journal of American Oil Chemists' Society 72(6): 661-664.
- Witthayapanyanon, A., Acosta, E. J., Harwell, J. H. and Sabatini, D. A. 2006. Formulation of ultralow interfacial tension systems using extended surfactants. Journal of Surfactants and Detergents 9: 331-339.
- Youngs, C. G. and Sallans, H. R. 1955. Acetone as a selective solvent for vegetable oils. Journal of American Oil Chemists' Society 32(7): 397-400.
- Zhu, Y., Xu, G., Gong, H., Wu, D. and Wang, Y. 2009. Production of ultra-low interfacial tension between crude oil and mixed brine solution of Triton X-100 and its oligomer Tyloxapol with cetyltrimethylammonium bromide induced by hydrolyzed polyacrylamide. Colloids and Surfaces A: Physicochem. Eng. Aspects 332: 90-97.

**CHAPTER 4. PAPER 2: DEVELOPMENT AND SCALE UP OF AQUEOUS  
SURFACTANT-ASSISTED EXTRACTION OF CANOLA OIL FOR USE AS  
BIODIESEL FEEDSTOCK**

Paper 2 is an edited and revised version of a poster presented at the ASABE Annual International Meeting in Dallas, TX, July 30-31 and August 1, 2012. Authors: Nattapong Tuntiwiwattanapun, Chantra Tongcumpou, Darrin Haagenson, and Dennis Wiesenborn. Title: Development and Scale Up of Aqueous Surfactant-assisted Extraction of Canola Oil for Use As a Biodiesel Feedstock. Session number: 221 (July 31, 2012). A revised version was submitted to *JAACS* as a manuscript on August 10, 2012. Nattapong Tuntiwiwattanapun, the author of this thesis, is the first author of Paper 2. He designed and conducted the experiments in this paper and also wrote Paper 2, which was edited by the other co-authors.

#### **4.1. Abstract**

Aqueous surfactant-assisted extraction (ASE) has been proposed as an alternative to hexane for extraction of vegetable oil; however, the use of inexpensive surfactants such as sodium dodecyl sulfate (SDS) and the effect of ASE on the quality of biodiesel from the oil are not well understood. Therefore, the effects on oil detachment efficiency of surfactant concentration, extraction time, oilseed to liquid ratio and other parameters were evaluated using ASE with ground canola and SDS in aqueous solution. The highest oil detachment efficiency was 80%, and was achieved using 0.02 M SDS at 20°C, solid-liquid ratio 1 : 10 (g : mL), 1,000 rpm stirring speed and 45 min contact time. Applying triple extraction with three stages reduced the amount of SDS solution needed by 50%. The ASE method was scaled up to extract 300 g of ground canola using the best combination of extraction conditions as described above. The extracted oil from the scale up of ASE method passed the recommendation for biodiesel feedstock quality with respect to water content, acid value and phosphorous content. Water content, kinematic viscosity, acid value and oxidative stability index of ASE biodiesel were within the ASTM D6751 biodiesel standards.

#### **4.2. Introduction**

Biodiesel is one of the renewable liquid fuels which can increase the world's energy security as well as reduce greenhouse gas emissions. Biodiesel is produced from vegetable oil and/or animal fat via transesterification. Canola oil has good potential to be an important biodiesel feedstock due to its high yield (1,500 to 2,200 kg/ha), high oil content (40-50%) and a suitable fatty acid profile for low temperature operation

(Gunstone, 2004). Content of free fatty acids, water and phospholipids are three important parameters for biodiesel feedstock quality. High content of free fatty acids and water can reduce biodiesel yield and quality by contributing to soap formation. Presence of phospholipids results in sediments which clog diesel engine filters (Knothe, Krahl and Van Gerpen, 2004). Many refining steps are required to make the oil or fat suitable for conversion to biodiesel.

In addition to feedstock quality concerns, there is a growing concern about the vegetable oil extraction process. Solvent extraction with hexane has been broadly used in the vegetable oil industry due to its high oil extraction efficiency, over 90%. However, the Clean Air Act (1990) categorized hexane as a hazardous air pollutant because of safety, health and environmental concerns. Thus, extraction systems must fully contain hexane and provide for a leak detection (Do and Sabatini, 2010). Future laws may be even more stringent; therefore, hexane-free extraction approaches are needed.

An aqueous extraction process (AEP), which uses water as the extraction medium, is one potential vegetable oil extraction method. Currently this method can achieve >70% oil extraction efficiency (Rosenthal, Pyle and Niranjan, 1996). However, to achieve this yield, the extractor must be operated at 50°C and a large amount of chemical reagent applied to adjust a pH to 12 (Rosenthal, Pyle and Niranjan, 1998).

One way to enhance AEP is to incorporate enzymes such as cellulase to hydrolyze plant cell walls, as well as protease to hydrolyze the protein network and assist demulsification. Since enzymes are selective to specific classes of compounds, a combination of enzymes is advantageous. This multiple enzyme-assisted aqueous

extraction yielded 65 to 75% oil (Dominguez, Ndfiez and Lema, 1994; Latif, Diosady and Anwar, 2008; Rosenthal et al., 1996). However, use of multiple enzymes further complicates optimization of reaction conditions such as pH and temperature. Recently, the yield of oil was increased to > 90% by applying flaking and extrusion as mechanical pre-treatments with use of a single proteolytic enzyme (de Moura et al., 2008; de Moura and Johnson, 2009; Lamsal and Johnson, 2007; Lamsal, Murphy and Johnson, 2006). These pre-treatments disrupt cell walls, which is a key to recovering oil (Rosenthal et al., 1996); thus, a cellulase enzyme is not necessary.

Another way to enhance AEP is to incorporate a surfactant, resulting in an operation known as aqueous surfactant-assisted extraction or “ASE”. A surfactant is an amphiphilic compound comprised of polar and non-polar parts, thus enabling the surfactant to reduce interfacial tension (IFT) of the system. By definition, IFT is the repulsive energy between two different species of molecules (Rosen, 2004). In other words, it is the minimum energy required to disperse one phase into another, increasing the surface area between the two phases. A high IFT value implies that the two species of molecules are very different in polarity.

The IFT value between water and vegetable oil is very high, between 20 and 30 mN/m (Gunstone, 2004). Therefore, in AEP much energy is required to detach vegetable oil from the oilseed surface and disperse it into aqueous phase. Reducing the IFT value by introducing surfactant eases the detachment and dispersion of the oil. Moreover, reduced IFT aids disruption of the oil trapped inside the oilseed matrix; the oil droplets are then readily transported to the aqueous phase (Campbell and Glatz, 2009).

Several reports showed that the IFT should be reduced to 0.01 mN/m (ultralow level) by incorporation of surfactant with co-solvent or use of an extended surfactant (Do and Sabatini, 2010; Do and Sabatini, 2011; Kadioglu, Phan and Sabatini, 2011; Naksuk, Sabatini and Tongcumpou, 2009). The extracted oil from ASE method has superior oil quality in terms of lower content of free fatty acids and phospholipids compared to that of hexane solvent extraction. However, the properties of biodiesel from this ASE extracted oil have not yet been reported. Moreover, using co-solvent, such as short chain alcohols, requires a leak-proof system; and the extended surfactant has not been commercialized yet. Do and Sabatini (Do and Sabatini, 2011) scaled this process up to extract 150 g of ground canola and peanut using an extended surfactant in the extraction medium; however, scaled-up with a commercially-available surfactant has not been reported.

The primary objectives of this work were: (1) using an inexpensive commercial surfactant without co-solvent as the extraction medium in ASE method of canola oil extraction; (2) maximizing the oil recovery by evaluating the effect of surfactant concentration, stirring and ultrasonication time, extraction temperature, solid-liquid ratio, and triple extraction with three stages; (3) scaling the process up to extract 300 g of ground canola for quality analysis and conversion to biodiesel; and (4) evaluating the biodiesel product quality to demonstrate the potential use of the extracted oil from the ASE method as a biodiesel feedstock. Furthermore, the biodiesel samples produced from screw press extraction, Soxhlet hexane extraction and commercial refined canola oil were also evaluated for quality.

### **4.3. Materials and Methods**

#### **4.3.1. Materials**

Sodium dodecyl sulfate (SDS, biotechnology grade) was purchased from AMRESCO® (Solon, OH). Methanol ( $\geq 99.8\%$  purity), hexane (95% purity) and KOH (99% purity) were purchased from EMD Millipore (Chemicals) (Billerica, MA). NaCl (99% purity) was purchased from BDH Chemical distributed by VWR International LLC (Chicago, IL). Commercial canola oil was purchased locally (Flavorite®, SUPERVALU International, Tacoma, WA). Canola seeds were kindly provided by Dr. Mukhles Rahman of the Plant Sciences Department of North Dakota State University.

#### **4.3.2. Oilseed pretreatment**

Prior to pretreatment, canola seeds were evaluated for moisture content by a grain moisture analyzer (model GAC 2100, DICKEY-john®, Auburn, IL). The moisture content was approximately 5 wt% (wet basis). Canola seeds were ground for 30 s using a Protor-Silex coffee grinder (model E160BY, Hamilton Beach Brands, Inc., Southern Pines, NC). Ground canola seed particles were then fractionated by using US sieve size No. 40 and 60 with Ro-Tap testing sieve shaker (The W.S. Tyler Company, Cleveland, OH). Only the fraction between 0.251 and 0.425 mm was used in this study, as recommended for oilseed extraction (Do and Sabatini, 2010; Kadioglu et al., 2011; Naksuk et al., 2009).

### 4.3.3. Aqueous surfactant-assisted extraction (ASE)

Ground canola (4 g) was suspended in 40 mL of 0.02 M SDS solution. The suspension was then mixed for 30 min at 1,000 rpm (13.9 kW-h/sample) on a PMC Data Plate® digital hot plate/stirrer model 730, and the slurry was then centrifuged 20 min at 2,000 X g (3,000 rpm; model CR412, Jouan, Inc., Winchester, VA). After centrifugation two fractions were obtained: liquid fraction (oil, emulsion, and extraction medium) and extracted meal. The liquid fraction was demulsified by heating to 70°C for 30 min. For quantification purposes only, hexane (50 mL) was then added to facilitate recovery of the extracted oil once the temperature was reduced to 30°C. The sample was allowed to stand in a separatory funnel for 10 min to separate the two immiscible phases. The bottom aqueous extraction medium was decanted; the upper organic phase (oil plus hexane) was desolventized at 105°C overnight and weighed.

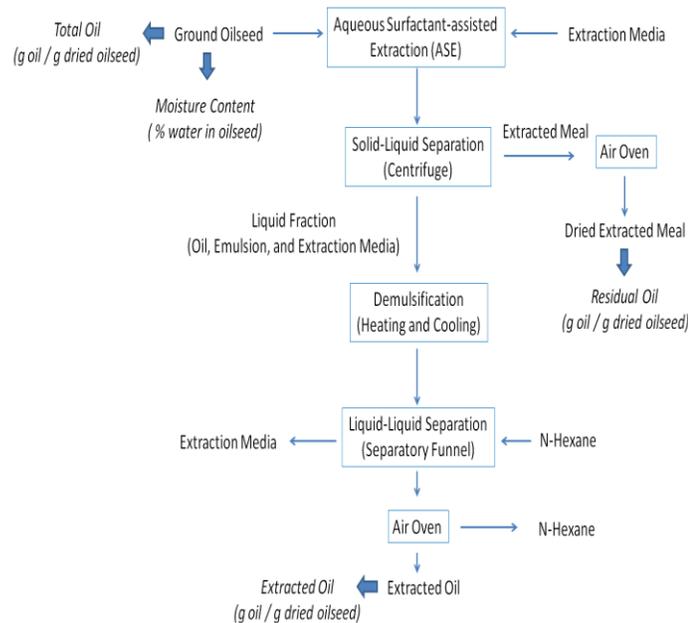


Figure 24. ASE method in this study with sample analyses in Paper 2.

#### 4.3.4. Oil content of oilseed and extracted meal

Crude oil was extracted from ground canola seed and residual oil from extracted canola meal via Soxhlet extractor, using hexane as solvent, as described in the AOCS official methods AM2-93 (American Society for Testing and Materials, 2009). The amounts of oil recovered were defined as the total oil (TO) and residual oil (RO) (g oil / g dried oilseed or meal) for ground seed and extracted meal, respectively. The “oil extraction efficiency” was calculated based on the weight of extracted oil (EO) from the extraction process relative to the total oil weight initially present in the ground seed sample (Equation 5a).

$$\text{Oil Extraction Efficiency (\%)} = \frac{EO}{TO} \times 100\% \quad (\text{Equation 5a})$$

However, in the case of ASE in this study, some extracted oil was lost to an emulsion, penalizing oil extraction efficiency. Thus, “oil detachment efficiency” (Equation 5b) was defined to better reflect the actual removal of oil from seed during extraction.

$$\text{Oil Detachment Efficiency (\%)} = \frac{(TO-RO)}{TO} \times 100\% \quad (\text{Equation 5b})$$

It should be noted that, with a very effective demulsification process, the values of oil extraction and oil detachment efficiency will be similar.

#### 4.3.5. Experimental design

**4.3.5.1. Effect of surfactant concentration.** The impact of aqueous SDS concentration on oil detachment and oil extraction efficiency was evaluated at four

concentrations: 0.005, 0.01, 0.02 and 0.03 M. The solid-liquid ratio was 1 g : 10 mL. A magnetic stirrer was applied for 30 min at 20°C. The SDS concentration that yielded the highest oil detachment and oil extraction efficiency was selected for the subsequent experiments.

**4.3.5.2. Effect of stirring time and ultrasonication.** The impact of magnetic stirring and ultrasonication time on oil detachment and oil extraction efficiency were investigated at four levels: 15, 30, 45 and 60 min. Ultrasonication was performed using an Aquasonic ultrasonic cleaner (11.4 kW-h/sample, 25 kHz, model 150 HT, VWR Scientific Product, West Chester, PA). Three glass beakers containing ground canola and SDS solution were placed in the ultrasonic bath with 4 L of distilled water. The SDS concentration determined from experiment 1 was applied with a solid-liquid ratio 1 g : 10 mL at 20°C for both the magnetic stirrer and ultrasonication bath. The extraction time that provided the highest oil detachment and oil extraction efficiency was selected for the subsequent experiments.

In addition, the effect of different ratios of magnetic stirrer and ultrasonication time on oil detachment efficiency was evaluated. The ratio of ultrasonication time : magnetic stirrer time was evaluated at five levels: 0:60, 15:45, 30:30, 45:15 and 60:0 (min:min). Use of the ultrasonication bath preceded the magnetic stirrer.

**4.3.5.3. Effect of extraction media and temperature.** Water and aqueous SDS solution (concentration determined in experiment 1) were compared as extraction media at three temperature levels: 20, 50 and 70°C. The solid-liquid ratio was 1 g : 10 mL with

stirring time determined from experiment 2. Also, hexane was used as a reference nonpolar solvent at 20°C.

**4.3.5.4. Effect of solid-liquid ratio.** The impact of solid-liquid ratio on oil detachment and oil extraction efficiency was evaluated at five levels: 1:30, 1:20, 1:10, 1:7 and 1:5 (g : mL). The SDS concentration and stirring time were selected from the previous experiments. The extraction temperature was 20°C.

**4.3.5.5. Effect of triple extraction with three stages.** The impact of triple extraction with three stages (3E3S) on oil detachment and oil extraction efficiency was investigated at three levels of solid-liquid ratio: 1:10, 1:7 and 1:5 (g : mL). The SDS concentration was selected from the previous experiment. The extraction temperature was 20°C. The stirring time of each extraction stage was 15 min. The three stages, denoted as A, B and C, were operated in six steps (Figure 25). After completion of a step, the liquid fraction (LF) from the previous extraction stage was used as extraction medium for the next stage. For example at solid-liquid ratio 1:10, each fresh SDS solution (FS), including FS 1, FS 2 and FS 3, was 40 mL with 4 g of ground canola in each stage. The liquid fractions from extraction Stage A, which were LF A1, LF A2 and LF A3, were used as extraction media for Stage B in Step 2, 3 and 4, respectively. The extracted meal from Stage A of Step 1 was re-extracted by using fresh SDS solution, FS 2 and FS 3 in Stage A of Step 2 and 3, respectively. The total liquid fraction (120 mL) and total extracted meal (40 g) were analyzed to determine the oil extraction and oil detachment efficiency, respectively, as described above.

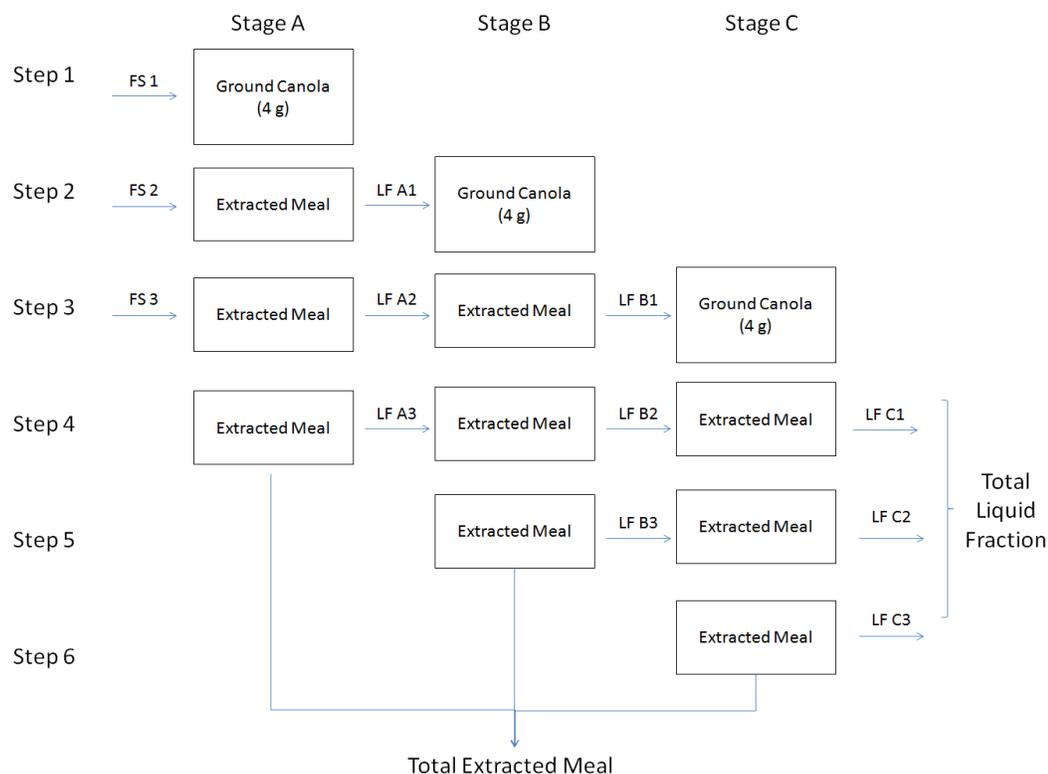


Figure 25. Diagram of triple extraction with three stages. FS was fresh SDS solution and LF was liquid fraction.

#### 4.3.6. Scale up of the ASE process

The batch size for the ASE process was increased to extract 300 g of ground canola using the best combination of extraction conditions (surfactant concentration, extraction time, extraction temperature and solid-liquid ratio) from the previous experiments. An agitator (model VW0708021, Arrow Engineering Co., Inc., Hillside, NJ) with a pitched- three blade impeller was used as a mixer at 1,000 rpm. The suspension was heated to 70°C for 30 min; and then allowed to stand for an additional 3 h. The upper layer, approximately 360 mL, was decanted off and centrifuged 45 min at 3,750 X g (4,000 rpm) to separate the crude oil from extraction medium. This extracted oil was

dried in a vacuum oven at 60°C, 80 kPa for 24 h before transferred to a dark amber bottle for further quality analysis transesterification.

#### **4.3.7. Other extraction methods**

A screw press (Komet Oil Expeller S 87G, IBGMonforts, Germany) was operated following the conditions of Haagensohn et al. (2010) with a press head temperature of 60°C. The screw press oil was transferred to a dark amber bottle without refining.

Soxhlet extraction with hexane was performed following the AOCS official methods AM2-93 (American Oil Chemists' Society, 1999) with 30 g of ground canola/batch using an extraction time of 4 h. The extracted oil was transferred to a dark amber bottle without refining.

#### **4.3.8. Transesterification**

The canola oil from the scaled-up ASE process, screw press and Soxhlet hexane extraction as well as commercial canola oil (200 g) were converted to biodiesel following the procedure of Haagensohn et al. (2010). The mole ratio of methanol : vegetable oil : KOH was 6.15 : 1 : 0.152. The finished biodiesel was transferred to a dark amber bottle for later quality analysis.

#### **4.3.9. Extracted oil and biodiesel quality analysis**

In order to evaluate the potential use of ASE extracted oil as a biodiesel feedstock, the oil from the scaled-up ASE process and biodiesel prepared from that oil were compared to those from screw press extraction, Soxhlet hexane extraction and commercial canola. Water content and acid value of the oil were determined following

the procedures of Haagenson et al. (2010). The fatty acid profiles of both extracted oil and biodiesel were analyzed by gas chromatography according to the method described by Vick et al. (Vick, Jan and Miller, 2004) and Espinoza-Perez et al. (Espinoza Perez et al., 2009). Water content, kinematic viscosity, cloud point, acid value and oxidative stability index of biodiesel were analyzed according to methods described in the ASTM D6751-09 for B100 (American Society for Testing and Materials, 2009). Phosphorous contents of extracted oils were quantified by inductively couple plasma (ICP) at Archer Daniels Midland (Enderlin, ND).

#### **4.3.10. Statistical analysis**

All extraction experiments were performed in triplicate. Error bars on charts were represent  $\pm 1$  SD. For quality analysis, each sample was analyzed in triplicate except phosphorous content with duplicate analysis. Data were analyzed using PROC ANOVA (SAS Institute, 2008). An F-protected LSD ( $P \leq 0.05$ ) was calculated for main effect mean comparisons. Significant differences ( $P \leq 0.05$ ) between means of two way and higher order interactions were determined as twice the standard error of the mean (Snedecor and Cochran, 1980).

### **4.4. Results and Discussion**

#### **4.4.1. Effect of surfactant concentration**

A previous study (Tuntiwiwattanapun, Wiesenborn and Tongcumpou, 2012) showed that SDS provided a lower IFT ( $< 1$  mN/m) with canola oil than two other commercial anionic surfactants: sodium bis(ethylhexyl) sulfosuccinate (AOT) and

sodium dihexyl sulfosuccinate (AMA). Increasing the SDS concentration from 0.005 M to 0.02 M resulted in an increase in both oil detachment and oil extraction efficiency, shown in Figure 26. This observation could be explained by a corresponding reduction in the IFT of the system (Do and Sabatini, 2010; Kadioglu et al., 2011; Naksuk et al., 2009).

At the critical micelle concentration (CMC) of each surfactant system, where the first micelle is formed, the IFT of the system is dramatically reduced (Rosen, 2004). In water, the CMC of SDS was 0.008 M at 25°C (Pierce, 2004). However, in heterogeneous systems (surfactant solution and oilseed particles), the surfactant monomers are adsorbed onto the particle surfaces. This reduces the IFT among the solid and two liquid phases (oil and water) leading to detachment of oil into the surfactant solution (Kadioglu et al., 2011; Naksuk et al., 2009). Consequently, in heterogeneous systems, the CMC is generally higher than the system without oilseed particles. Thus, it can be expected that the CMC of SDS in the extraction process should probably be higher than 0.008 M at 25°C. The oil detachment and oil extraction efficiency achieved with 0.01 M SDS was significantly lower than that with 0.02 M SDS.

However, a further increase in concentration beyond 0.02 M SDS decreased oil extraction efficiency because of reduced recovery of the detached oil which was dissolved into the micelles. As a consequence, the oil detachment efficiency at 0.03 M SDS was not significantly different from that at 0.02 M SDS, but the oil extraction efficiency was significantly lower (Figure 26). Therefore, the results indicated that the CMC was between 0.01 M and 0.02 M SDS, and the 0.02 M SDS solution was selected for subsequent experiments.

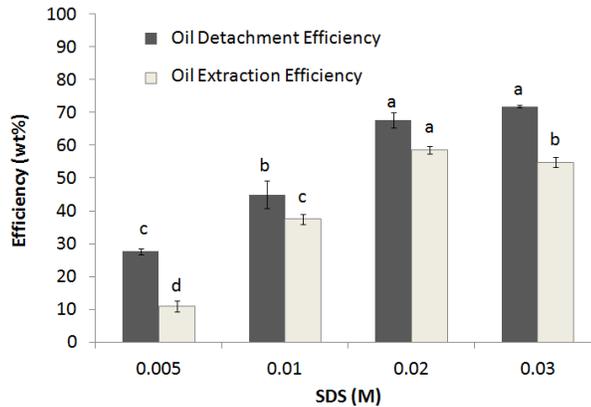


Figure 26. Effect of SDS concentration on oil detachment and oil extraction efficiency at 20°C with solid-liquid ratio 1 g : 10 mL, stirring for 30 min. Means within the bar graph followed by the same color and letter are not significantly different at  $P \leq 0.05$ .

#### 4.4.2. Effect of stirring time and ultrasonication

There was no increase in oil extraction or oil detachment efficiency after 45 min extraction time with a magnetic stirrer (Figure 27A). Similar results were observed using an ultrasonication bath (Figure 27B) with a similar power input (kW-h/sample). Increasing extraction time from 30 min to 45 min significantly increased oil detachment and oil extraction efficiency. One explanation may be a reduction of IFT during the extraction time. A preliminary study (data not shown) found the IFT steadily decreased after up to 30 min contact time and then become stable. Thus, that study might imply that the highest oil detachment and oil extraction efficiency should be achieved after 30 min extraction time. Ultrasonication provided a much lower oil extraction efficiency compared to that of magnetic stirrer at all four extraction times. This result might come from the hydrodynamic shear-force which was generated from air bubble cavitation by ultrasonic waves. This high shear force could enhance the emulsion formation, reducing oil extraction efficiency while still achieving high oil detachment efficiency.

The combination of ultrasonication and stirring on oil detachment efficiency was also investigated. However, there was no significant difference in oil detachment efficiency among these ratios as shown in Appendix B (Table 20). As a result of the above mixing study, subsequent extractions were carried out using the magnetic stirrer for 45 min. This resulted in the highest oil detachment efficiency, 80%.

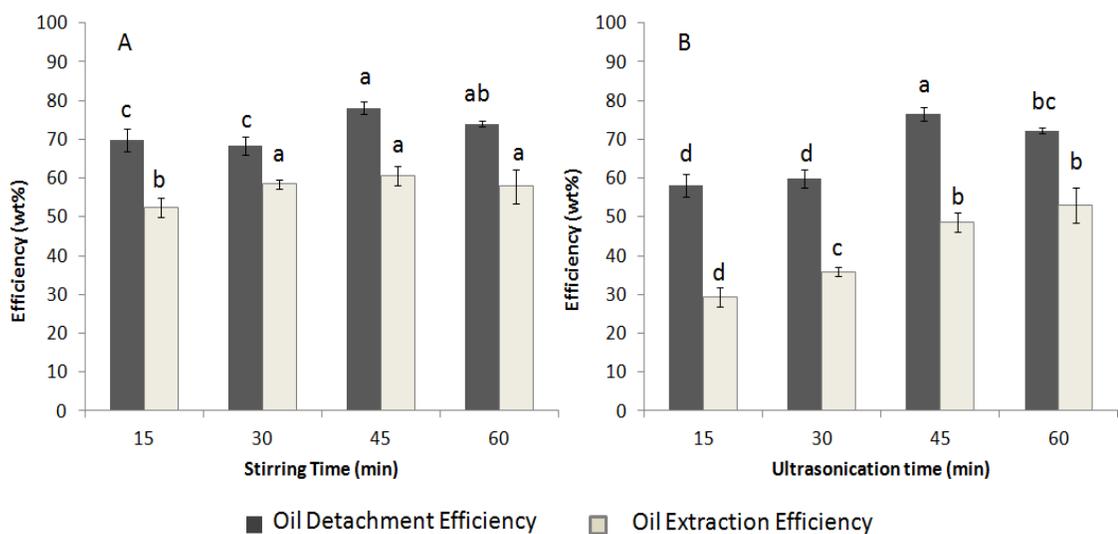


Figure 27. Effect of extraction time on oil detachment and oil extraction efficiency for 0.02 M SDS solution at 20°C with solid-liquid ratio 1 g : 10 mL when mixing with (a) a magnetic stirrer and (b) an ultrasonication bath. Means within the bar graph of both (a) and (b) followed by the same color and letter are not significantly different at  $P \leq 0.05$ .

#### 4.4.3. Effect of extraction media and temperature

Increasing extraction temperature to 70°C improved the oil detachment efficiency when water without surfactant was used as the extraction medium (Figure 28). In contrast, there was no significant impact of temperature on oil detachment efficiency in the case of SDS solution. This is due to the fact that anionic surfactants such as SDS are generally insensitive to temperature (Rosen, 2004). Surfactant solution provided much higher oil detachment efficiency than water at every extraction temperature. The highest oil

detachment efficiency achieved by using water was approximately half that of the SDS solution. This result could be explained by the IFT of the system. The IFT between water and vegetable oil is between 20 and 30 mN/m (Do and Sabatini, 2010; Gunstone, 2004), while the IFT between canola oil and SDS solution in this work was  $< 1$  mN/m (Tuntiwiwattanapun et al., 2012). Therefore, surfactant solution reduced the heat energy required to disperse canola oil into the extraction medium. In addition, the oil detachment efficiency for SDS solution at any temperature treatment was not significantly different from that of hexane at 20°C (Figure 28). These results suggest the use of 0.02 M SDS solution as an alternative extraction medium to hexane for extraction at room temperature. However, it should be noted that the vegetable oil industry commonly uses hexane at 60 to 65°C with the oil extraction efficiency exceeding 95% (Anderson, 2011; Beckel, Belter and Smith, 1946; Kemper, 1997).

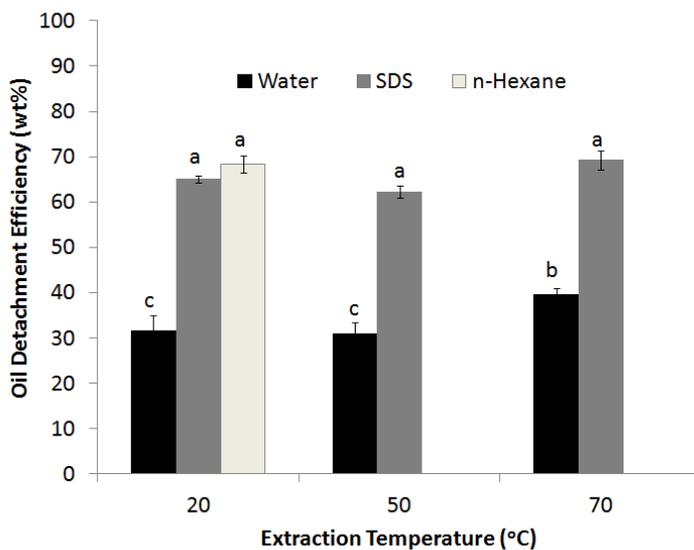


Figure 28. Oil detachment efficiency versus extraction temperature using water, 0.02 M SDS solution and *n*-hexane as extraction medium with solid-liquid ratio 1 g : 10 mL, stirring for 45 min. Canola particle size was larger than 0.425 mm. Means within the bar graph followed by the same letter are not significantly different at  $P \leq 0.05$ .

#### 4.4.4. Effect of solid-liquid ratio

Increasing solid-liquid ratio to higher than 1 g : 10 mL substantially reduced oil detachment and oil extraction efficiency, because there was insufficient surfactant in the system (Figure 29). Reducing the solid to liquid ratio from 1 g : 10 mL to 1 g : 30 mL had no impact on oil detachment efficiency, but the oil extraction was decreased. This may be similar to the “effect of surfactant concentration” in which an increase in SDS concentration from 0.02 M to 0.03 M resulted in a larger disparity between oil detachment and oil extraction efficiency. As explained above, increased surfactant mass in the system may increase emulsion formation.

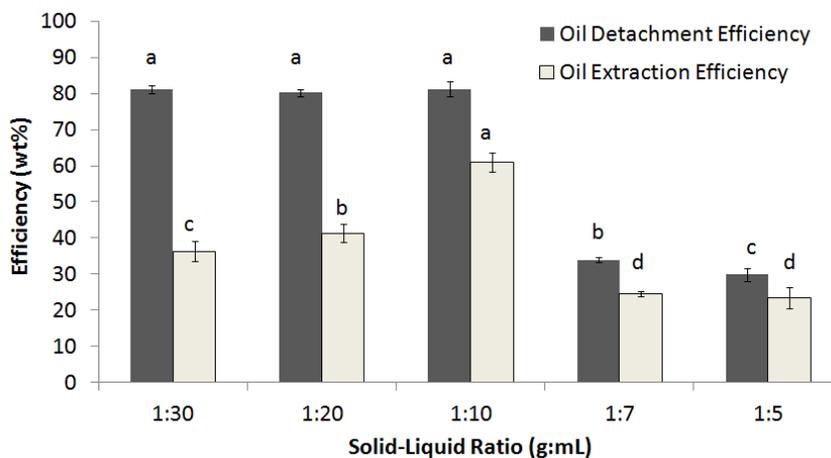


Figure 29. Impact of solid-liquid ratio on oil detachment and oil extraction efficiency for 0.02 M SDS solution at 20°C, stirring for 45 min. Means within the bar graph followed by the same color and letter are not significantly different at  $P \leq 0.05$ .

#### 4.4.5. Effect of triple extraction with three stages (3E3S)

Applying 3E3S improved the oil detachment and oil extraction efficiency at a high level of solid -liquid ratio. Increasing the solid-liquid ratio slightly decreased oil

detachment efficiency, but the oil extraction efficiency of 3E3S at each solid-liquid ratio was not significantly different (Figure 30 and 31). At solid-liquid ratios higher than 1 g : 10 mL; however, the 3E3S provided higher oil detachment and oil extraction efficiency than the standard ASE method (single extraction). Do and Sabatini (Do and Sabatini, 2011) also reported that an additional ASE extraction stage increased the oil extraction efficiency of canola and peanut oil, even when using deionized water alone as the extraction medium in the additional stage. Rosenthal et al. (Rosenthal et al., 1998) showed that the extraction of protein and oil increased with the number of extraction stages in the AEP method. One explanation was solid-liquid mass-transfer mechanisms. In the ASE method, the oil extraction followed dispersion and/or diffusion kinetics. After the first extraction stage, extracted oil in micelle was dispersed in surfactant solution. The extracted meal was then re-extracted by fresh surfactant solution or reused surfactant solution. The oil concentration in extraction medium of additional step must be lower than that of the first extraction stage. Another explanation was the meal retained some of the emulsion from the first stage. Thus, the additional stage may be simply was away this residual emulsion. Therefore, the solid-liquid mass-transfer mechanisms were then enhanced, resulting in higher oil extraction efficiency. As a result, 3E3S reduced the volume of surfactant usage by 50% versus single extraction and one stage, with 80 and 60% oil detachment and oil extraction efficiency, respectively.

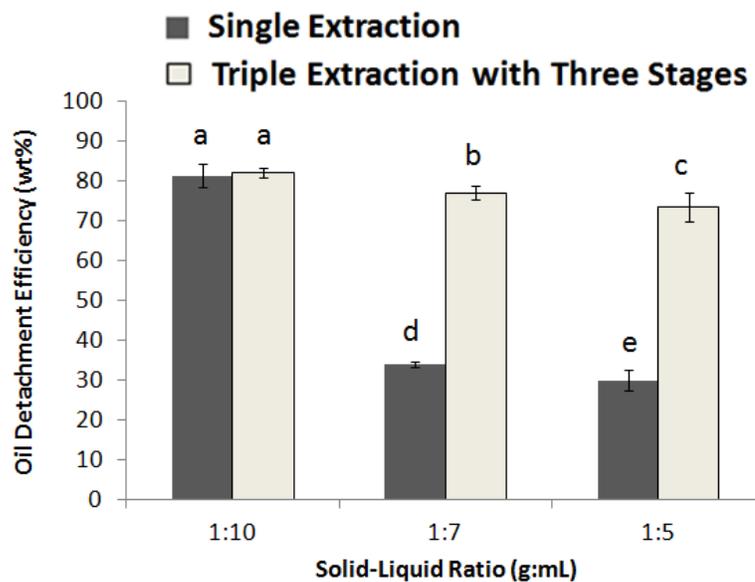


Figure 30. Impact of triple extraction with three stages on oil detachment efficiency for 0.02 M SDS solution at 20°C, stirring for total contact time 45 min. Means within the bar graph followed by the same letter are not significantly different at  $P \leq 0.05$ .

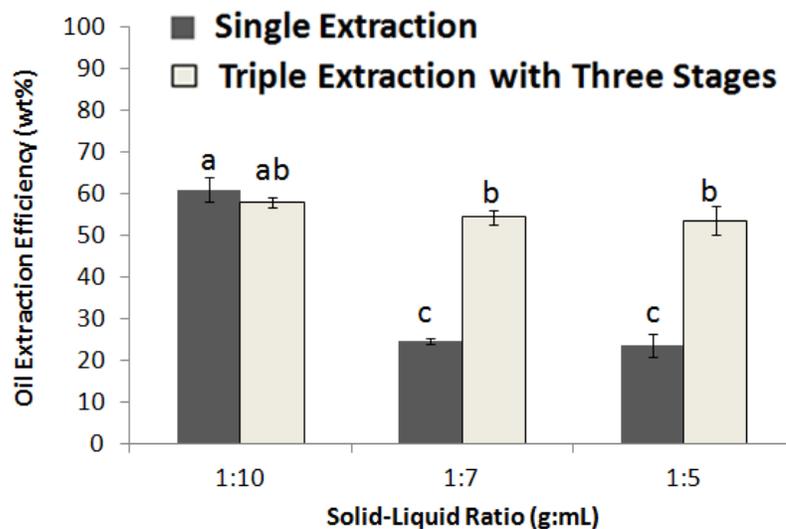


Figure 31. Impact of triple extraction with three stages on extraction efficiency for 0.02 M SDS solution at 20°C, stirring for total contact time 45 min. Means within the bar graph followed by the same letter are not significantly different at  $P \leq 0.05$ .

#### 4.4.6. Scale up of the ASE process

Oil from ground canola (300 g) was extracted via the ASE process using the best combination of extraction conditions determined from the previous experiments: 0.02 M SDS solution, 45 min extraction time, 20°C and solid-liquid ratio 1 g : 10 mL. The oil extraction efficiency was only 40%, but could be readily improved through centrifugation of the full mixture of extracted canola meal and extraction medium (solid/liquid separation). Do and Sabatini (Do and Sabatini, 2011) applied a semi-continuous solid/liquid centrifuge (4,114 X g) to separate extracted meal from extraction medium, and then used a liquid/liquid centrifuge (680 X g) to separate vegetable oil from aqueous solution. The oil extraction efficiency from the dual centrifuges exceeded 90%. Moreover, the solid/liquid centrifuge might reduce the adsorption of extracted oil on the surface of extraction unit. The scaled-up ASE process is illustrated in Figure 32.

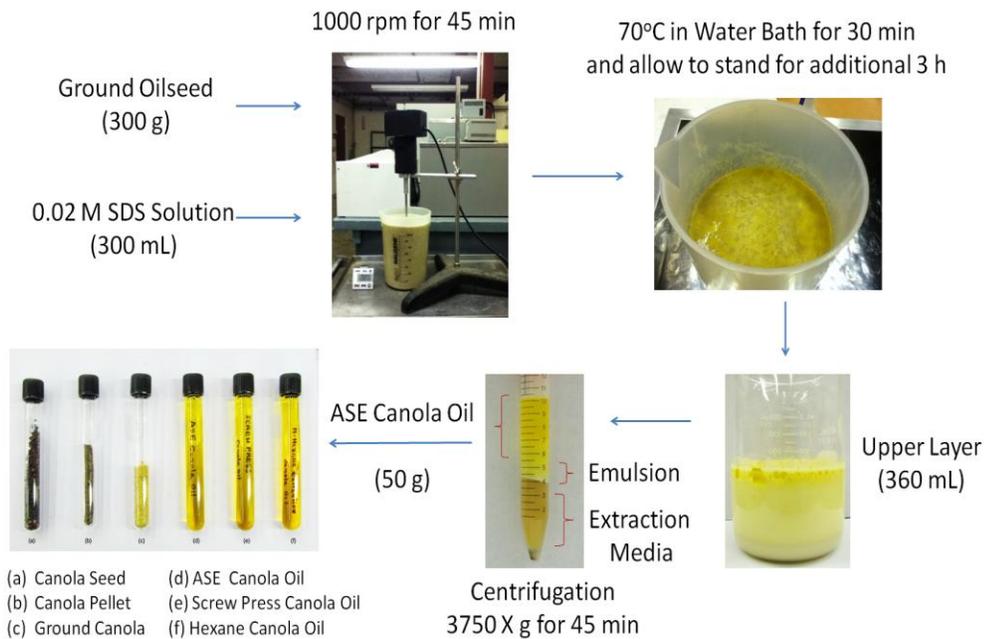


Figure 32. Diagram of scaled-up ASE process.

Table 7. Comparison of extraction conditions and oil efficiency between references and this work.

Reference	Naksuk et al. (2009)	Do and Sabatini (2010)	Kadioglu et al. (2011)	Results and conditions in this thesis
Oilseed and sample size	Palm kernel (1g)	Peanut and Canola (2 g)	Corn germ (4 g)	Canola (4 g)
Surfactant system	Comperland KD (3 wt%)	C10-18PO-2EOsulfate (0.15 wt%)	C12,14-10PO-2EOsulfate (0.4 wt%)	SDS (0.02 M)
	Alfoterra 145- 5PO (0.1 wt%)	NaCl (6 wt%) for peanut	NaCl (1 wt%)	or SDS (0.58 wt%)
	NaCl (10 wt%)	NaCl (5 wt%) for canola		
IFT (mN/m)	< 0.01	< 0.01	< 0.01	< 1
Pretreatment	Grinding	Food processor	Food processor	Coffee mill
	Dehulling	Yes	Yes	Not Reported
	Size Selection	US Sieve size No.40-70 (0.21-0.42 mm)	US Sieve size No.40-70 (0.21-0.42 mm)	US Sieve size No.40-70 (0.21-0.42 mm)
	Thermal Treatment	Not Reported	104 °C with 35 min	Yes (dried corn germ)
Extraction	Seed-Liquid ratio	1 to 10 (g/mL)	2 to 10 (g/g)	1 to 10 (g/g)
	Extraction	Not Reported	Horizontal shaker 150 cycle/min	Horizontal shaker 250 cycle/min
	Duration	with 30 min	with 30 min	with 45 min
	Separation	Not Reported	Centrifuge speed 2,170 g with 30 min	Centrifuge speed 3,500 rpm with 20 min
Oil Extraction Efficiency	93.99% (based on soxhlet extraction)	93-95 % (based on soxhlet extraction)	83% (based on hexane solvent in ASE)	80 % detachment efficiency 60% extraction efficiency

#### **4.4.7. Extracted oil quality**

The extracted canola oil samples from the scaled-up ASE process, screw press, and Soxhlet extraction were analyzed for biodiesel feedstock quality, specifically water content, acid value, and phosphorus content, and compared with commercial canola oil.

**4.4.7.1. Water content.** The presence of water in vegetable oil leads to poor biodiesel yield and quality by contributing to soap formation (Van Gerpen and Knothe, 2004). All samples passed the recommended limit (Table 8).

**4.4.7.2. Acid value.** The acid value indicates the free fatty acid content in biodiesel feedstock from the hydrolysis of triglyceride. The high content of free fatty acid reduces the biodiesel performance, because it reacts with basic catalysts to form soap. All extracted oils in this study were below the recommended upper limit, despite the absence of a caustic refining process step (Table 8). Therefore, hydrolysis of triglycerides during ASE process was not a problem. Moreover, the extracted oil by ASE had a lower acid value than that from Soxhlet extraction. The higher acid value from Soxhlet extraction may be due to triglyceride hydrolysis under elevated temperature with long extraction time. Similar results were reported by other investigators (Do and Sabatini, 2010; Dunford and Su, 2010; Hanmoungjai, Pyle and Niranjana, 2000; Kadioglu et al., 2011; Latif et al., 2008).

**4.4.7.3. Phosphorus content.** The presence of phospholipids (>300 ppm) has a potential to generate an emulsion during transesterification, resulting in decreased biodiesel yield and quality (Ahn et al., 1995; Van Gerpen and Dvorak, 2002). All samples passed the recommendation for phosphorous content (Table 8). The low

phosphorus contents of unrefined extracted oil might be due to the precipitation of phospholipids during the three weeks of storage time prior to analysis.

#### **4.4.8. Biodiesel quality**

The extracted canola oils from the scaled-up ASE process, screw press, Soxhlet extraction, and the commercial canola oil were converted to biodiesel via alkaline transesterification. The resulting canola biodiesels were analyzed for quality, including water content, kinematic viscosity, cloud point, acid value, and oxidative stability index.

**4.4.8.1. Water content.** Presence of water in biodiesel is a critical issue since it tends to cause poor combustion, filter plugging and smoking (Fernando et al., 2007) as well as reduced storage life (American Society for Testing and Materials, 2009). The water content of biofuel in this work, except biodiesel from commercial oil, passed the standard (Table 8). The explanation for high water content of biodiesel derived from commercial oil is not clear, but it might be due to insufficient vacuum drying.

**4.4.8.2. Kinematic viscosity.** Biodiesel viscosity is decreased when compared to vegetable oil, and kinematic viscosity is used to monitor the degree of transesterification. De Filippis et al. (1995) and Fernando et al. (2007) reported that improved transesterification efficiency was associated with lower kinematic viscosity. All biodiesel samples passed the standard; and the kinematic viscosity of biodiesel from commercial oil was higher than that derived from other canola oil feed stocks (Table 8).

**4.4.8.3. Cloud point.** Cloud point is the temperature at which the first formation of wax crystals is observed in biodiesel (American Society for Testing and Materials,

2009). This property is primarily related to the fatty acid profile of the biodiesel feedstock and cloud point temperatures ranging from 1 to  $-2^{\circ}\text{C}$  have been reported previously for canola (Gunstone, 2004; Haagensohn et al., 2010; Kulkarni, Dalai and Bakhshi, 2006). Each biodiesel sample in this study had very similar fatty acid composition, as shown in Table 25 in Appendix B. Thus their cloud points were not expected to be different. Surprisingly, the cloud points of biodiesel from three extracted oils were  $< -6^{\circ}\text{C}$ ; while that of biodiesel from commercial oil was approximately  $-2^{\circ}\text{C}$  (Table 8). The cause of the decreased cloud point temperature is not clear, but these results showed that all biodiesel samples in this study could be suitable in low temperature conditions.

**4.4.8.4. Acid value.** Biodiesel possessing high acid values may cause diesel engine corrosion as well as increased fuel deposits, increasing filter plugging potential (American Society for Testing and Materials, 2009). The acid value of each biodiesel sample was well within the standard (Table 8) and was lower than its feedstock, except the biodiesel from commercial oil. The reduction of the acid value in the biodiesel product might come from the reaction of base catalyst (KOH) with free fatty acids in vegetable oil during transesterification, contributing to soap formation.

**4.4.8.5. Oxidative stability index (OSI).** This value indicates the susceptibility of biodiesel to oxidation at high temperature and presence of air. Biodiesel with low OSI may generate sediments, increased acid values, and higher viscosity over the course of storage. This can plug fuel filters and reduce engine performance by plugging combustor nozzles or injectors (American Society for Testing and Materials, 2009). All biodiesel

samples in this study passed the standard and the OSI derived from commercial canola oil was significantly poorer than the biodiesel from ASE, screw press and Soxhlet extraction. Like cloud point temperature, OSI is a function of the biodiesel feedstock fatty acid profile. However, the fatty acid profiles of sample were not different. Differences in OSI of the biodiesel could partly be attributed to differences in the content of natural antioxidants such as tocopherols, which were not quantified. Haas and Scott (Hass and Scott, 2007) found that the concentration of tocopherols in soy biodiesel derived from refined oil was 76% lower than that of *in situ* transesterification (simultaneous vegetable oil extraction and transesterification). They speculated that biodiesel from the *in situ* transesterification process should have a higher OSI than conventional transesterification which used refined oil as biodiesel feedstock. Haagenson et al. (Haagenson et al., 2010) confirmed that the OSI of biodiesel from *in situ* transesterification was higher than canola biodiesel from refined oil. The refined oil was exposed to high temperatures, especially during moisture removal after refining.

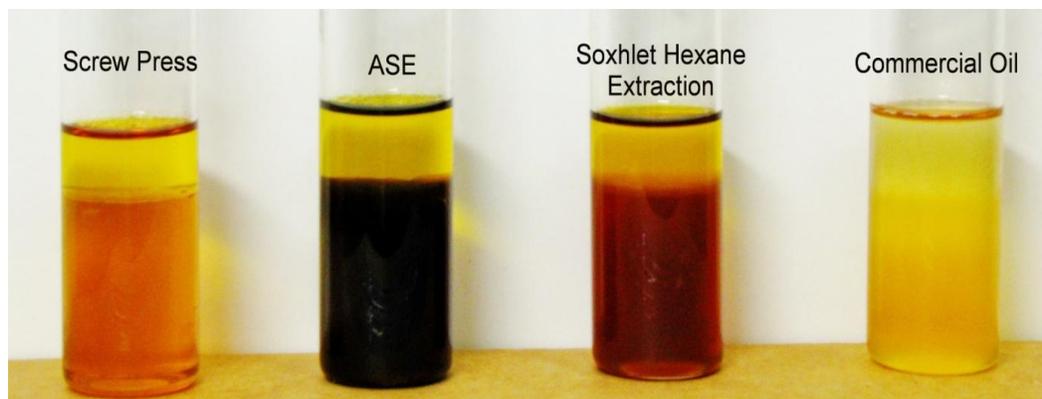


Figure 33. Canola biodiesel (upper phase) and glycerol (lower phase) from three extracted oils and commercial oil.

Table 8. Extracted oil and biodiesel product quality.

	Parameter	Extraction Method			Commercial Oil	Recommended Limit
		Screw Press	ASE	Soxhlet <i>n</i> -Hexane		
Extracted Oil	Water content (vol%)	0.040 a	0.033 c	0.036 b	0.006 d	0.05 <sup>A</sup>
	Acid value (mg KOH/g)	0.28 c	0.33 b	0.41 a	0.09 d	0.5 <sup>A</sup>
	Phosphorous Content (ppm)	10	19	49	8	300 <sup>B</sup>
	Parameter	Screw Press Oil	ASE Oil	Soxhlet Oil	Commercial Oil	ASTM D6751
Biodiesel	Water content (vol%)	0.035 b	0.026 c	0.034 b	0.060 a	<0.05
	Kinematic viscosity (mm <sup>2</sup> /s)	5.23 b	5.14 b	5.25 b	5.42 a	1.9-6.0
	Cloud point (°C)	-7 b	-6 b	-6 b	-2 a	Report
	Acid value (mg KOH/g)	0.19 d	0.25 c	0.30 b	0.34 a	<0.5
	Oxidative stability index (h)	8.67 a	6.10 b	7.02 ab	3.17 c	>3

Means within each row followed by the same letter are not significantly different at  $P < 0.05$ .

<sup>A</sup> and <sup>B</sup> Recommended upper limit of vegetable oil to be used as biodiesel feedstock by (Van Gerpen and Knothe, 2004) and (Ahn et al., 1995), respectively.

#### 4.5. Conclusions

The ASE method offered several advantages when compared with hexane extraction. ASE method incorporated non-toxic chemicals, thus minimizing pollutant emission and waste generation as well as reduced energy consumption since it was able to operate at room temperature. Surfactant concentration, extraction time, and solid to liquid ratio significantly impacted on oil detachment and oil extraction efficiency. The highest oil detachment and oil extraction efficiency were 80 and 60%, respectively, using SDS 0.02 M at 20°C with solid-liquid ratio 1 g : 10 mL, stirring 1,000 rpm and 45 min contact time. The IFT in this work was 100 times higher than the recommendation level, but high shear force generated from magnetic stirrer might compensate this high IFT

value. Moreover, applying 3E3S reduced the use of extraction medium. However, the critical problem of ASE method in this study was emulsion formation.

The extracted oil from ASE method had superior oil quality to that of Soxhlet extraction in terms of lower acid value and phospholipids content which are the important parameters for biodiesel feedstock quality. The biodiesel from ASE oil passed the ASTM D6751 standards for water content, kinematic viscosity, acid value and OSI. The cloud point was below  $-6^{\circ}\text{C}$ , thus it was suitable for operation in cold temperatures. Hence, the extracted canola oil from ASE method may be suitable for use as a biodiesel feedstock.

#### **4.6. Acknowledgements**

The authors are thankful for the research support from the North Dakota Agricultural Experiment Station and the North Dakota Center of Excellence for Oilseed Development as well as the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, the Center of Excellence for Environmental and Hazardous Waste Management (Thailand). Dr. Mukhlesur Rahman, canola breeder in the Department of Plant Sciences at North Dakota State University, is also acknowledged for providing canola seeds. The gas chromatography analysis provided by Leonard Cook, (USDA-ARS, Fargo, ND) and inductively coupled plasma analysis for quantifying phosphorous content by Archer Daniels Midland (Enderlin, ND) are gratefully acknowledged.

#### **4.7. References**

Ahn, E., Koncar, M., Mittelbach, M. and Marr, R. 1995. A low-waste process for the production of biodiesel. Separation Science and Technology 30: 2021-2033.

- American Oil Chemists' Society. 1999. "Oil content in oilseeds." AOCS method AM2-93. Champaign, IL: AOCS.
- American Society for Testing and Materials. 2009. "Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels." ASTM D6751-09. West Conshohocken: ASTM.
- Anderson, G. E. Edible oil processing: solvent extraction. [Online]. 2011. AOCS. Available from: <http://lipidlibrary.aocs.org/processing/solventextract/index.htm> [20 August 2012]
- Beckel, A. C., Belter, P. A. and Smith, A. K. 1946. Laboratory study of continuous vegetable oil extraction: countercurrent extractor, rising-film evaporator, and oil stripper. Industrial and Engineering Chemistry 18(1): 56-58.
- Campbell, K. A. and Glatz, C. E. 2009. Mechanisms of aqueous extraction of soybean oil. Agricultural and Food Chemistry 57: 10904-10912.
- De Filippis, P., Giavarini, C., Scarsella, M. and Sorrentino, M. 1995. Transesterification processes for vegetable oils: a simple control method of methyl ester content. Journal of American Oil Chemists' Society 72: 1399-1404.
- de Moura, J. M. L. N., Campbell, K. A., Mahfuz, A., Jung, S., Glatz, C. E. and Johnson, L. 2008. Enzyme-assisted aqueous extraction of oil and protein from soybeans and cream de-emulsification. Journal of American Oil Chemists' Society 85: 985-995.
- de Moura, J. M. L. N. and Johnson, L. A. 2009. Two-stage countercurrent enzyme-assisted aqueous extraction processing of oil and protein from soybeans. Journal of American Oil Chemists' Society 86: 283-289.

- Do, L. D. and Sabatini, D. A. 2010. Aqueous extended-surfactant based method for vegetable oil extraction: proof of concept. Journal of American Oil Chemists' Society 87: 1211-1220.
- Do, L. D. and Sabatini, D. A. 2011. Pilot scale study of vegetable oil extraction by surfactant-assisted aqueous extraction process. Separation Science and Technology 46(6): 978-985.
- Dominguez, H., Ndfiez, M. J. and Lema, J. M. 1994. Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: a review. Journal of Food Chemistry 49: 271-286.
- Dunford, N. T. and Su, A. 2010. Effect of canola oil quality on biodiesel conversion efficiency and properties. Transaction of the ASABE 53(3): 993-997.
- Espinoza Perez, J. D., Haagenson, D., Pryor, S. W., Ulven, C. A. and Wiesenborn, D. 2009. Production and characterization of epoxidized canola oil. Transaction of the ASAE 52(4): 1-9.
- Fernando, S., Karra, P., Hernandez, R. and Jha, S. K. 2007. Effect of incompletely converted soybean oil on biodiesel quality. Energy 32: 844-851.
- Gunstone, F. D. 2004. Rapeseed and canola oil: production, processing, properties and uses. Florida: CRS Press.
- Haagenson, D. M., Brudvik, R. L., Lin, H. and Wiesenborn, D. P. 2010. Implementing an in situ alkaline transesterification method for canola biodiesel quality screening. Journal of American Oil Chemists' Society 87: 1351-1358.
- Hanmoungjai, P., Pyle, L. and Niranjana, K. 2000. Extraction of rice bran oil using aqueous media. Journal of Chemical Technology and Biotechnology 75: 348-352.

- Hass, M. J. and Scott, K. M. 2007. Moisture removal substantially improves the efficiency of in situ biodiesel production from soybeans. Journal of American Oil Chemists' Society 81: 83-89.
- Kadioglu, S. I., Phan, T. T. and Sabatini, D. A. 2011. Surfactant-based oil extraction of corn germ. Journal of American Oil Chemists' Society 88: 863-869.
- Kemper, T. G. Extraction principles and extractor design. 1997. In P. J. Wan and P. J. Wakelyn (eds.), Technology and solvents for extracting oilseeds and nonpetroleum oils, pp.137-141. Champaign, IL: AOCS Press.
- Knothe, G., Krahl, J. and Van Gerpen, J. 2004. The biodiesel handbook. Champaign: AOCS Press.
- Kulkarni, M. G., Dalai, A. K. and Bakhshi, N. N. 2006. Utilization of green seed canola oil for biodiesel production. Chemical Technology and Biotechnology 81: 1886-1893.
- Lamsal, B. P. and Johnson, L. A. 2007. Separating oil from aqueous extraction fractions of soybean. Journal of American Oil Chemists' Society 84: 785-792.
- Lamsal, B. P., Murphy, P. A. and Johnson, L. A. 2006. Flaking and extrusion as mechanical treatments for enzyme-assisted aqueous extraction of oil from soybeans. Journal of American Oil Chemists' Society 83: 973-979.
- Latif, S., Diosady, L. L. and Anwar, F. 2008. Enzyme-assisted aqueous extraction of oil and protein from canola (*Brassica napus* L.) seeds. European Journal of Lipid Science and Technology 110: 887-892.

- Naksuk, A., Sabatini, D. A. and Tongcumpou, C. 2009. Microemulsion-based palm kernel oil extraction using mixed surfactant solutions. Industrial Crops and Products 30: 194-198.
- Pierce. Technical resource: Remove detergent from protein samples. [Online]. 2004. Pierce Biotechnology, Inc. Available from: [http://wolfson.huji.ac.il/purification/PDF/detergents/PIERCE\\_DetergentRemoval.pdf](http://wolfson.huji.ac.il/purification/PDF/detergents/PIERCE_DetergentRemoval.pdf) [14 May 2012]
- Rosen, M. J. 2004. Surfactant and Interfacial Phenomena. 2nd ed. New York: John Wiley & Sons.
- Rosenthal, A., Pyle, D. L. and Niranjana, K. 1996. Aqueous and enzymatic process for edible oil extraction. Enzyme and Microbial Technology 19: 402-420.
- Rosenthal, A., Pyle, D. L. and Niranjana, K. 1998. Simultaneous aqueous extraction of oil and protein from soybean: Mechanisms for process design. Transaction of Institution of Chemical Engineers 76(C): 224-230.
- SAS Institute. 2008. "Release version 9.2 for window." Cary: SAS Institute.
- Snedecor, G. W. and Cochran, W. G. 1980. Statistical Methods. 7th ed. Ames: Iowa State University Press.
- Tuntiwiwattanapun, N., Wiesenborn, D. and Tongcumpou, C. 2012. "Optimization of vegetable oil detachment efficiency by reducing interfacial tension." 2012 ASABE/CSBE North Central Intersectional Conference. Fargo, ND: ASABE, RRV12113.

- Van Gerpen, J. and Dvorak, B. 2002. "The effect of phosphorus level on the total glycerol and reaction yield of biodiesel." The 10th Biennial Bioenergy Conference. Boise, ID.
- Van Gerpen, J. and Knothe, G. Basic of the transesterification reaction. 2004. In G. Knothe, J. Krahl and J. Van Gerpen (eds.), The biodiesel handbook, pp.26-41. Champaign: AOCS Press.
- Vick, B. A., Jan, C. C. and Miller, J. F. 2004. Two-year study on the inheritance of reduced saturated fatty acid content in sunflower seed. Helia 27(41): 25-40.

## CHAPTER 5. GENERAL CONCLUSIONS AND FUTURE WORK

### 5.1. General Conclusions

A commercial anionic surfactant solution (SDS) has potential use as an extraction medium in the ASE method of canola oil extraction. The addition of the 0.01 M nonionic surfactant LS-series and introduction of NaCl were found to improve the dynamic IFT of the system. However, surfactant precipitation was a limitation to introducing NaCl into the system. Even though the dynamic IFT was 10 times higher than the suggested level (0.01 mN/m), a promising level of oil detachment efficiency (> 70%) was achieved. The other studies on the ASE method showed a correlation between dynamic IFT and oil extraction efficiency. However, there was no significant difference in this study in oil detachment efficiency between the conditions that provided the lowest dynamic IFT (0.148 mN/m) and the highest dynamic IFT (0.869 mN/m). This may imply that, though dynamic IFT of the system plays an important role in oil detachment efficiency, other parameters such as mixing conditions may also affect the extraction performance.

Surfactant concentration, extraction time, and solid-liquid ratio had an impact on oil detachment and oil extraction efficiency in the ASE method. Meanwhile, there was no effect of extraction temperature on oil detachment and oil extraction efficiency in the case of 0.02 M SDS solution. The highest oil detachment and oil extraction efficiency were 80 and 60%, respectively, using 0.02 M SDS at 20°C with a solid-liquid ratio of 1 g : 10 mL, stirring speed of 1,000 rpm and contact time of 45 min. The ASE method offers several advantages over hexane extraction. The ASE method used non-toxic chemicals in the process, thus minimizing pollutant emission and waste generation. Energy

consumption was reduced since it was able to operate at room temperature. Moreover, the extraction media could be reduced to 5 mL : 1 g of ground canola when triple extraction with three stages was applied.

The extracted oil from the ASE method showed potential to be used as a biodiesel feedstock. It provided superior quality to that of Soxhlet hexane extraction in terms of lower free fatty acid and phosphorous content. The biodiesel from the ASE oil passed the ASTM D6751 standards in water content, kinematic viscosity, acid value and oxidative stability index.

## **5.2. Future Work**

The ASE method is a promising alternative to hexane for vegetable oil extraction, since extraction can be accomplished in the absence of any toxic chemicals and operated at room temperature. However, one problem of this method was emulsion formation leading to low oil extraction efficiency, 60%. The performance of demulsification process in this study was not adequate, resulting in the 20% gap between oil detachment and oil extraction efficiency. Optimization of demulsification conditions, including temperature, centrifugation, introducing demulsifying agent, and applying high voltage, would increase the oil extraction efficiency of ASE method. Moreover, surfactant recovery processes, such as precipitation by electrolyte and cloud point separation by temperature, should be investigated in the future work.

In addition to vegetable oil extraction, surfactant solution is also applied in protein extraction. Thus, development of simultaneous extraction of vegetable oil and protein may help this process become commercially viable.

Evaluation of surfactants in transesterification would be worthwhile. The surfactant might reduce the amount of methanol and KOH needed as well as the reaction time, due to increased solubilization and surface area (See “Optimize Biodiesel Production and Quality” in the *Literature Review* section). Moreover, this knowledge could be applied to *in situ* transesterification. *In situ* transesterification is the simultaneous extraction and transesterification of the vegetable oil. The surfactant could accelerate the process time for the following reasons. Methanol, alone, has limited ability to extract the oil trapped inside the oilseed matrix. Surfactant can disrupt the trapped oil into droplets small enough for dispersing into methanol solution. Surfactant may also increase the transesterification rate, since surface area will be increased by reducing IFT.

**APPENDIX A. EXPERIMENTAL DATA OF DYNAMIC INTERFACIAL  
TENSION VALUE**

Table A1. Surfactant properties and structure.

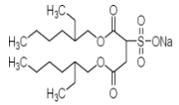
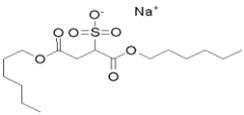
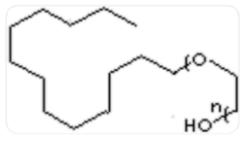
Surfactant	Type	HLB	MW (g/mol)	Molecular Formula	Molecular Structure
AOT	anionic	10.5	444.56	$C_{20}H_{37}NaO_7S$	
AMA	anionic	14	388	$C_{16}H_{29}NaO_7S$	
SDS	anionic	40	288.38	$NaC_{12}H_{25}SO_4$	
Dehydol LS-1	nonionic	3.6	244	$C_{15}H_{32}O_2$	
Dehydol LS-2	nonionic	6.1	288	$C_{17}H_{36}O_3$	
Dehydol LS-3	nonionic	7.9	332	$C_{19}H_{40}O_4$	
Dehydol LS-9	nonionic	13.4	596	$C_{31}H_{64}O_{10}$	

Table A2. Dynamic IFT of three commercial anionic surfactants and canola oil.

Replication	Anionic Surfactant (1% wt)	Vegetable Oil	IFT (mN/m)	Mean
1	AOT	Canola	1.371	1.452 b
2	AOT	Canola	1.462	
3	AOT	Canola	1.522	
1	AMA	Canola	4.586	4.436 a
2	AMA	Canola	4.465	
3	AMA	Canola	4.257	
1	SDS	Canola	0.839	0.836 c
2	SDS	Canola	0.84	
3	SDS	Canola	0.828	

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table A3. Dynamic IFT of mixed surfactant systems and canola oil.

Replication	Anionic Surfactant (0.03M)	Nonionic Surfactant (0.01 M)	Vegetable Oil	IFT (mN/m)	Mean
1	SDS	-	Canola	0.839	0.836 b
2	SDS	-	Canola	0.84	
3	SDS	-	Canola	0.828	
1	SDS	LS-1	Canola	0.618	0.610 d
2	SDS	LS-1	Canola	0.61	
3	SDS	LS-1	Canola	0.601	
1	SDS	LS-2	Canola	0.764	0.771 c
2	SDS	LS-2	Canola	0.769	
3	SDS	LS-2	Canola	0.781	
1	SDS	LS-3	Canola	0.764	0.766 c
2	SDS	LS-3	Canola	0.769	
3	SDS	LS-3	Canola	0.766	
1	SDS	LS-9	Canola	1.128	1.136 a
2	SDS	LS-9	Canola	1.133	
3	SDS	LS-9	Canola	1.147	

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table A4. Dynamic IFT between canola oil and 0.03M SDS solution with various NaCl concentrations.

Replication	Anionic Surfactant (0.03M)	Nonionic Surfactant (0.01 M)	NaCl (M)	IFT (mN/m)	Mean
1	SDS	-	0	0.859	0.869 a
2	SDS	-	0	0.885	
3	SDS	-	0	0.862	
1	SDS	-	0.05	0.688	0.675 b
2	SDS	-	0.05	0.675	
3	SDS	-	0.05	0.663	
1	SDS	-	0.1	0.633	0.637 c
2	SDS	-	0.1	0.636	
3	SDS	-	0.1	0.641	
1	SDS	-	0.15	0.641	0.622 c
2	SDS	-	0.15	0.598	
3	SDS	-	0.15	0.626	
1	SDS	-	0.2	0.618	0.61 c
2	SDS	-	0.2	0.591	
3	SDS	-	0.2	0.622	
1	SDS	-	0.25	0.285	0.296 d
2	SDS	-	0.25	0.297	
3	SDS	-	0.25	0.306	
1	SDS	-	0.3	0.277	0.271 d
2	SDS	-	0.3	0.278	
3	SDS	-	0.3	0.258	
1	SDS	-	0.325	0.148	0.148 g
2	SDS	-	0.325	0.147	
3	SDS	-	0.325	0.149	

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table A4. Dynamic IFT between canola oil and 0.03M SDS solution with various NaCl concentrations (continued).

Replication	Anionic Surfactant (0.03M)	Nonionic Surfactant (0.01 M)	NaCl (M)	IFT (mN/m)	Mean
1	SDS	-	0.35	0.178	0.188 f
2	SDS	-	0.35	0.194	
3	SDS	-	0.35	0.191	
1	SDS	-	0.375	0.248	0.261 e
2	SDS	-	0.375	0.265	
3	SDS	-	0.375	0.271	
1	SDS	-	0.4	0.26	0.254 e
2	SDS	-	0.4	0.244	
3	SDS	-	0.4	0.257	
1	SDS	-	0.45	0.245	0.249 e
2	SDS	-	0.45	0.249	
3	SDS	-	0.45	0.253	
1	SDS	-	0.5	0.245	0.248 e
2	SDS	-	0.5	0.254	
3	SDS	-	0.5	0.246	

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table A5. Dynamic IFT of canola oil and mixed surfactant solution consisted of 0.03M SDS and 0.01M LS-1 at various NaCl concentrations.

Replication	Anionic Surfactant (0.03 M)	Nonionic Surfactant (0.01 M)	NaCl (M)	IFT (mN/m)	Mean
1	SDS	LS-1	0	0.618	0.610 a
2	SDS	LS-1	0	0.61	
3	SDS	LS-1	0	0.601	
1	SDS	LS-1	0.01	0.587	0.592 b
2	SDS	LS-1	0.01	0.591	
3	SDS	LS-1	0.01	0.598	
1	SDS	LS-1	0.02	0.592	0.587 b
2	SDS	LS-1	0.02	0.589	
3	SDS	LS-1	0.02	0.579	
1	SDS	LS-1	0.03	0.569	0.565 c
2	SDS	LS-1	0.03	0.561	
3	SDS	LS-1	0.03	0.566	
1	SDS	LS-1	0.05	0.574	0.568 c
2	SDS	LS-1	0.05	0.57	
3	SDS	LS-1	0.05	0.559	
1	SDS	LS-1	0.1	Precipitated	
2	SDS	LS-1	0.1	Precipitated	
3	SDS	LS-1	0.1	Precipitated	

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table A6. Dynamic IFT of canola oil and mixed surfactant solution consisted of 0.03M SDS and 0.01M LS-2 with various NaCl concentrations.

Replication	Anionic Surfactant (0.03M)	Nonionic Surfactant (0.01M)	NaCl (M)	IFT (mN/m)	Mean
1	SDS	LS-2	0	0.764	0.771 a
2	SDS	LS-2	0	0.769	
3	SDS	LS-2	0	0.781	
1	SDS	LS-2	0.05	0.638	0.626 b
2	SDS	LS-2	0.05	0.618	
3	SDS	LS-2	0.05	0.622	
1	SDS	LS-2	0.1	0.578	0.571 c
2	SDS	LS-2	0.1	0.566	
3	SDS	LS-2	0.1	0.57	
1	SDS	LS-2	0.15	0.586	0.574 c
2	SDS	LS-2	0.15	0.571	
3	SDS	LS-2	0.15	0.564	
1	SDS	LS-2	0.2	0.477	0.477 d
2	SDS	LS-2	0.2	0.465	
3	SDS	LS-2	0.2	0.488	
1	SDS	LS-2	0.25	0.465	0.467 d
2	SDS	LS-2	0.25	0.474	
3	SDS	LS-2	0.25	0.463	
1	SDS	LS-2	0.3	0.473	0.473 d
2	SDS	LS-2	0.3	0.48	
3	SDS	LS-2	0.3	0.466	
1	SDS	LS-2	0.325	0.465	0.469 d
2	SDS	LS-2	0.325	0.447	
3	SDS	LS-2	0.325	0.495	
1	SDS	LS-2	0.35	Precipitated	
2	SDS	LS-2	0.35	Precipitated	
3	SDS	LS-2	0.35	Precipitated	

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table A7. Dynamic IFT of canola oil and mixed surfactant solution consisted of 0.03M SDS and 0.01M LS-3 with various NaCl concentrations.

Replication	Anionic Surfactant (0.03 M)	Nonionic Surfactant (0.01 M)	NaCl (M)	IFT (mN/m)	Mean
1	SDS	LS-3	0	0.764	0.766 a
2	SDS	LS-3	0	0.769	
3	SDS	LS-3	0	0.766	
1	SDS	LS-3	0.025	0.633	0.644 e
2	SDS	LS-3	0.025	0.654	
3	SDS	LS-3	0.025	0.644	
1	SDS	LS-3	0.05	0.674	0.676 d
2	SDS	LS-3	0.05	0.669	
3	SDS	LS-3	0.05	0.684	
1	SDS	LS-3	0.1	0.687	0.692 c
2	SDS	LS-3	0.1	0.69	
3	SDS	LS-3	0.1	0.699	
1	SDS	LS-3	0.15	0.712	0.711 b
2	SDS	LS-3	0.15	0.706	
3	SDS	LS-3	0.15	0.714	
1	SDS	LS-3	0.25	0.683	0.694 c
2	SDS	LS-3	0.25	0.699	
3	SDS	LS-3	0.25	0.701	
1	SDS	LS-3	0.3	0.633	0.644 e
2	SDS	LS-3	0.3	0.654	
3	SDS	LS-3	0.3	0.644	
1	SDS	LS-3	0.325	0.624	0.629 f
2	SDS	LS-3	0.325	0.634	
3	SDS	LS-3	0.325	0.628	
1	SDS	LS-3	0.35	0.599	0.602 g
2	SDS	LS-3	0.35	0.596	
3	SDS	LS-3	0.35	0.611	
1	SDS	LS-3	0.4	0.624	0.629 f
2	SDS	LS-3	0.4	0.634	
3	SDS	LS-3	0.4	0.628	
1	SDS	LS-3	0.45	Precipitated	
2	SDS	LS-3	0.45	Precipitated	
3	SDS	LS-3	0.45	Precipitated	

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table A8. Dynamic IFT and oil detachment efficiency (OD) of ASE canola oil extraction using 0.03M SDS solution with various NaCl concentrations.

Replication	NaCl (M)	IFT (mN/m)	Mean	OD (%)	Mean
1	0.000	0.859	0.869 a	81.33	80.72 a
2	0.000	0.885		81.24	
3	0.000	0.862		79.58	
1	0.050	0.688	0.675 b	78.28	76.33 abc
2	0.050	0.675		75.67	
3	0.050	0.663		75.04	
1	0.150	0.641	0.622 c	75.42	75.64 cd
2	0.150	0.598		75.67	
3	0.150	0.626		75.82	
1	0.250	0.285	0.296 d	75.68	74.91 d
2	0.250	0.297		74.43	
3	0.250	0.306		74.61	
1	0.325	0.148	0.148 f	74.36	78.32 abc
2	0.325	0.147		81.41	
3	0.325	0.149		79.2	
1	0.375	0.248	0.261 e	76.15	75.79 cd
2	0.375	0.265		75.23	
3	0.375	0.271		75.98	
1	0.400	0.26	0.254 e	78.3	78.83 ab
2	0.400	0.244		79.41	
3	0.400	0.257		78.77	

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

## APPENDIX B. ASE EXTRACTION CONDITIONS AND EXPERIMENTAL

### DATA

Table B1. ASE conditions of each experiment in Paper 2.

	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
Surfactant Concentration (M)	0.005, 0.01, 0.02, 0.03	Selected	Selected	Selected	Selected
Extraction Time (min)	30	15, 30, 45, 60	Selected	Selected	15
Extraction Temperature (°C)	20	20	20, 50, 70	20	20
ASE Extraction	Single	Single	Single	Single	Triple Extraction with Three-stages
Solid-Liquid Ratio (g : mL)	1:10	1:10	1:10	1:30, 1:20, 1:10, 1:7, 1:5	1:5, 1:7, 1:10
Extraction Method	Stirring	Stirring, Ultrasonication	Stirring	Stirring	Stirring

Table B2. Oil detachment (OD) and oil extraction (OE) efficiency of ASE method with various SDS concentrations (Experiment 1, Paper 2).

Treatment (4)		Raw Data		Mean	
Replication	SDS Conc. (M)	OD (%)	OE (%)	OD (%)	OE (%)
1	0.005	27.62	9.94	27.99 c	10.96 d
2	0.005	27.27	10.18		
3	0.005	29.09	12.76		
1	0.01	38.15	39.02	43.02 b	37.45 c
2	0.01	45.92	37.43		
3	0.01	44.98	35.89		
1	0.02	70.93	58.91	68.33 a	58.48 a
2	0.02	67.55	57.20		
3	0.02	66.52	59.34		
1	0.03	71.97	53.12	71.74 a	54.84 b
2	0.03	71.42	56.22		
3	0.03	71.84	55.19		

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table B3. Oil detachment (OD) and oil extraction (OE) efficiency of ASE method using magnetic stirrer and ultrasonication bath with various extraction times (Experiment 2, Paper 2).

Replication	Treatment (2X4)		Raw Data		Mean	
	Mixing method	Extraction Time (min)	OD (%)	OE (%)	OD (%)	OE (%)
1	Stirring	15	69.49	51.58	69.76 c	52.38 b
2	Stirring	15	66.97	50.36		
3	Stirring	15	72.82	55.18		
1	Stirring	30	70.93	58.91	68.34 c	58.48 a
2	Stirring	30	67.56	57.20		
3	Stirring	30	66.53	59.33		
1	Stirring	45	80.01	63.55	78.13 a	60.65 a
2	Stirring	45	76.80	59.81		
3	Stirring	45	77.59	58.60		
1	Stirring	60	73.67	55.18	74.00 ab	57.84 a
2	Stirring	60	73.56	55.32		
3	Stirring	60	74.78	63.03		
1	Ultrasonication	15	55.54	30.57	58.21 d	29.26 d
2	Ultrasonication	15	61.19	30.91		
3	Ultrasonication	15	57.90	26.31		
1	Ultrasonication	30	59.07	33.65	59.79 d	35.88 c
2	Ultrasonication	30	59.16	38.25		
3	Ultrasonication	30	61.14	35.72		
1	Ultrasonication	45	72.30	46.19	76.57 a	48.65 b
2	Ultrasonication	45	79.08	49.96		
3	Ultrasonication	45	78.33	49.81		
1	Ultrasonication	60	71.29	52.59	72.21 bc	52.94 b
2	Ultrasonication	60	74.06	52.96		
3	Ultrasonication	60	71.28	53.28		

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table B4. Oil detachment (OD) and oil extraction (OE) efficiency of ASE method using extraction combination between ultrasonication and stirring with various extraction time ratios (Experiment 2, Paper 2).

Treatment (5)		Raw Data		Mean	
Replication	Extraction time ratio of Ultrasonication to Stirring (min:min)	OD (%)	OE (%)	OD (%)	OE (%)
1	0:60	73.67	55.18	74.00 a	57.84 ab
2	0:60	73.56	55.32		
3	0:60	74.78	63.03		
1	15:45	68.42	52.84	72.23 a	59.27 a
2	15:45	74.26	62.39		
3	15:45	74.02	62.59		
1	30:30	72.79	56.27	73.12 a	55.90 ab
2	30:30	73.72	57.24		
3	30:30	72.85	54.20		
1	45:15	73.10	43.94	73.53 a	45.39 c
2	45:15	74.05	46.81		
3	45:15	73.44	45.41		
1	60:0	71.29	52.59	72.21 a	52.94 b
2	60:0	74.06	52.96		
3	60:0	71.28	53.28		

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table B5. Oil detachment (OD) efficiency at 20°C of ASE method oil using water, 0.02M SDS and n-hexane as extraction medium (Experiment 3, Paper 2).

Treatment (3)		OD (%)	
Replication	Extraction Media	Raw Data	Mean
1	Water	31.10	31.62 b
2	Water	28.58	
3	Water	35.17	
1	SDS	65.70	65.05 a
2	SDS	64.32	
3	SDS	65.14	
1	<i>n</i> -Hexane	66.44	68.32 a
2	<i>n</i> -Hexane	70.24	
3	<i>n</i> -Hexane	68.28	

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table B6. Oil detachment (OD) efficiency of ASE method using water and 0.02M SDS solution as extraction medium with various extraction temperatures (Experiment 3, Paper 2).

Treatment (2X3)			OD (%)	
Replication	Extraction Media	Extraction Temperature (°C)	Raw Data	Mean
1	Water	20	31.10	31.62 d
2	Water	20	28.58	
3	Water	20	35.17	
1	Water	50	30.83	31.05 d
2	Water	50	28.79	
3	Water	50	33.52	
1	Water	70	41.26	39.63 c
2	Water	70	39.05	
3	Water	70	38.59	
1	SDS	20	65.70	65.05 ab
2	SDS	20	64.32	
3	SDS	20	65.14	
1	SDS	50	63.86	62.34 b
2	SDS	50	61.57	
3	SDS	50	61.61	
1	SDS	70	66.92	69.30 a
2	SDS	70	70.40	
3	SDS	70	70.57	

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table B7. Oil detachment (OD) and oil extraction (OE) efficiency of ASE method with various solid-liquid ratios (Experiment 4, Paper 2).

Replication	Treatment (5)	Raw Data		Mean	
	Solid to Liquid (g : mL)	OD (%)	OE (%)	OD (%)	OE (%)
1	1:5	31.90	25.88	29.89 c	23.50 d
2	1:5	28.82	20.15		
3	1:5	28.94	24.46		
1	1:7	33.79	24.88	33.95 b	24.59 d
2	1:7	33.39	23.89		
3	1:7	34.67	25.02		
1	1:10	81.17	60.97	81.30 a	60.88 a
2	1:10	79.30	63.46		
3	1:10	83.43	58.21		
1	1:20	81.00	43.53	80.21 a	41.32 b
2	1:20	79.08	38.58		
3	1:20	80.54	41.85		
1	1:30	80.51	39.34	81.15 a	36.33 c
2	1:30	82.32	33.93		
3	1:30	80.63	35.71		

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table B8. Oil detachment (OD) and oil extraction (OE) efficiency of ASE method using triple extraction with three stages (3E3S) with various solid-liquid ratios (Experiment 5, Paper 2).

Replication	Treatment (2X3)		Raw Data		Mean	
	No of Extraction	Solid to Liquid (g : mL)	OD (%)	OE (%)	OD (%)	OE (%)
1	Single	1:5	31.90	25.88	29.89 e	23.50 c
2	Single	1:5	28.82	20.15		
3	Single	1:5	28.94	24.46		
1	Single	1:7	33.79	24.88	33.95 d	24.59 c
2	Single	1:7	33.39	23.89		
3	Single	1:7	34.67	25.02		
1	Single	1:10	81.17	60.97	81.30 a	60.88 a
2	Single	1:10	79.30	63.46		
3	Single	1:10	83.43	58.21		
1	3E3S	1:5	74.44	52.49	73.36 c	53.53 b
2	3E3S	1:5	72.43	54.99		
3	3E3S	1:5	73.21	53.10		
1	3E3S	1:7	78.32	52.47	76.91 b	54.33 b
2	3E3S	1:7	75.86	55.72		
3	3E3S	1:7	76.55	54.80		
1	3E3S	1:10	83.53	57.86	82.06 a	57.80 ab
2	3E3S	1:10	80.02	54.29		
3	3E3S	1:10	82.62	61.24		

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

**APPENDIX C. EXPERIMENTAL DATA OF EXTRACTED CANOLA OIL AND  
BIODIESEL QUALITY**

Table C1. Fatty acid profile of three extracted canola oils and commercial canola oil.

Canola Oil	Fatty Acid (% wt)				
	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
Screw Press Extraction	4.6	1.8	64.0	19.4	8.7
ASE	4.8	1.8	65.2	19.6	8.6
Soxhlet <i>n</i> -Hexane Extraction	4.9	1.8	65.0	19.8	8.6
Commercial Oil	4.6	1.9	64.6	19.9	9.0

Table C2. Water content and acid value of extracted canola oil and commercial canola oil.

Replication	Canola Oil	Raw Data		Mean	
		Water Content (vol%)	Acid Value (mg KOH/g)	Water Content (vol%)	Acid Value (mg KOH/g)
1	Screw Press	0.41	0.26	0.40 a	0.28 c
2	Screw Press	0.40	0.29		
3	Screw Press	0.39	0.28		
1	ASE	0.33	0.33	0.33 c	0.33 b
2	ASE	0.32	0.36		
3	ASE	0.33	0.30		
1	Soxhlet <i>n</i> -Hexane	0.35	0.39	0.36 b	0.41 a
2	Soxhlet <i>n</i> -Hexane	0.35	0.43		
3	Soxhlet <i>n</i> -Hexane	0.36	0.41		
1	Commercial Oil	0.06	0.08	0.06 d	0.10 d
2	Commercial Oil	0.06	0.09		
3	Commercial Oil	0.06	0.12		

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table C3. Water and soap content of canola biodiesel from three extracted oils and commercial oil.

Replication	Biodiesel	Raw Data		Mean	
		Water Content (vol%)	Soap Content (ppm)	Water Content (vol%)	Soap Content (ppm)
1	Screw Press	0.36	847	0.35 b	848 b
2	Screw Press	0.35	833		
3	Screw Press	0.35	865		
1	ASE	0.25	540	0.26 c	535 c
2	ASE	0.26	504		
3	ASE	0.27	561		
1	Soxhlet <i>n</i> -Hexane	0.34	525	0.34 b	510 c
2	Soxhlet <i>n</i> -Hexane	0.34	494		
3	Soxhlet <i>n</i> -Hexane	0.33	510		
1	Commercial Oil	0.60	897	0.60 a	943 a
2	Commercial Oil	0.61	943		
3	Commercial Oil	0.59	990		

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table C4. Kinematic viscosity and cloud point of canola biodiesel from three extracted oils and commercial oil.

Replication	Biodiesel	Raw Data		Mean	
		Kinematic Viscosity (mm <sup>2</sup> /s)	Cloud Point (°C)	Kinematic Viscosity (mm <sup>2</sup> /s)	Cloud Point (°C)
1	Screw Press	5.18	-7	5.23 b	-7 b
2	Screw Press	5.27	-7		
3	Screw Press	5.22	-6		
1	ASE	5.14	-7	5.14 b	-6 b
2	ASE	5.13	-6		
3	ASE	5.14	-6		
1	Soxhlet <i>n</i> -Hexane	5.25	-6	5.25 b	-6 b
2	Soxhlet <i>n</i> -Hexane	5.25	-6		
3	Soxhlet <i>n</i> -Hexane	5.25	-7		
1	Commercial Oil	5.49	-3	5.42 a	-2 a
2	Commercial Oil	5.28	-2		
3	Commercial Oil	5.49	-2		

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .

Table C5. Acid value and oxidative stability of canola biodiesel from three extracted oils and commercial oil.

Replication	Biodiesel	Raw Data		Mean	
		Acid Value (mg KOH/g)	Oxidative Stability (h)	Acid Value (mg KOH/g)	Oxidative Stability (h)
1	Screw Press	0.20	7.25	0.19 d	8.67 a
2	Screw Press	0.19	9.55		
3	Screw Press	0.19	9.20		
1	ASE	0.24	6.50	0.25 c	6.10 b
2	ASE	0.24	7.10		
3	ASE	0.26	4.70		
1	Soxhlet <i>n</i> -Hexane	0.27	8.05	0.30 b	7.02 ab
2	Soxhlet <i>n</i> -Hexane	0.29	6.50		
3	Soxhlet <i>n</i> -Hexane	0.34	6.50		
1	Commercial Oil	0.32	4.30	0.34 a	3.17 c
2	Commercial Oil	0.36	3.10		
3	Commercial Oil	0.36	2.10		

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ .