EXTRICATION OF BIODIESEL FEEDSTOCK FROM EARLY STAGE OF FOOD WASTE LIQUEFACTION

MARIDAH BINTI MOHD AMIN

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF TECHNOLOGY
(ENVIRONMENTAL MANAGEMENT)

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2015

ABSTRACT

This study aims to provide alternative solution in the management of domestic waste and the possibility of using the extracted free fatty acid (FFA) in the food waste as the feedstock for the production of biodiesel. The main concern of acquiring the FFA from the food waste is the challenge which lies during its extraction due to high water content. Prior to the pre-treatment of food waste via the natural biological cycle, the selection of best FFA extraction method was performed on the fresh food waste. It was found that the use of Reflux method is more effective compared to the Soxhlet method. Both extraction methods used two solvents, dichloromethane and n-hexane. Despite of using the different solvent, similar profile FFA and amount have been observed during both extractions. The effectiveness of choosing Reflux method is because it does not require the drying process, when compared to Soxhlet. The following experimentation was carried out to extract the FFA on the hydrolysed food waste during its liquefaction process. This experiment used two types of hydrolysed food waste, natural liquefaction and enhanced liquefaction process, with and without introduction of seed sludge, respectively. Both liquefaction processes had undergone beyond the hydrolysis stage, which has included the acidification stage. Results have shown that the natural liquefaction of food waste had provided higher yield of FFA, specifically in 72 hours liquefaction stage. In a different experiment, major FFA profiles which were obtained during the natural 72 hours of liquefaction process was transesterification and found to produced highly unsaturated fatty acid methyl ester (FAME) ranging from C16 to C18. Nevertheless when the transesterification of such FFA using C2-C5 modelled compound, the process had produced the opposite profiling of FAME, which mainly saturated. Therefore, natural liquefaction of food waste has the potential to produce the feedstock for biodiesel.

Keywords: food waste, free fatty acid, extrication, biodiesel, esterification

ABSTRAK

Penyelidikan ini dijalankan bagi memberi kaedah alternatif kepada pengurusan sisa buangan tempatan dan kemungkinan untuk mengekstrak asid lemak bebas dalam sisa bahan buangan makanan untuk pembuatan biodiesel. Masalah utama dalam pengekstrakan asid lemak bebas daripada sisa bahan buangan makanan adalah kandungan air yang tinggi. Sebelum kaedah rawatan awal melalui kitar biologi semulajadi, pemilihan kaedah terbaik pengekstrakan asid lemak bebas dijalankan ke atas sisa bahan buangan makanan. Didapati kaedah *Reflux* adalah lebih efektif berbanding kaedah *Sokhlet*. Kedua-dua kaedah pengekstrakan menggunakan dua jenis pelarut iaitu *dicloromethane* dan *n-hexane*. Walaupun dua jenis pelarut berbeza digunakan, didapati keduanya menunjukkan senarai dan kandungan asid lemak bebas yang sama. Pemilihan kaedah Reflux dipilh adalah kerana kaedah ini tidak memerlukan peringkat pengeringan. Eksperimen yang seterusnya telah dijalankan untuk mengekstrak asid lemak bebas pada sisa bahan makanan buangan hidrolisis semasa proses pencairannya. Eksperiman ini telah menggunakan dua jenis sisa bahan buangan makanan hidrolisis, iaitu pencairan semulajadi dan proses pencairan yang telah dipertingkatkan di mana masing-masing diperkenalkan dan tidak diperkenalkan kepada sisa kumbahan. Kedua-dua proses pencairan telah melalui proses tahap hidrolisis melampau, yang melibatkan peringkat asidifikasi. Keputusan menunjukkan pencairan semulajadi bahan buangan makanan menghasilkan kandungan asid lemak yang tinggi terutama selepas 72 jam. Dalam eksperiman yang lain, asid lemak utama diperolehi ketika bahan 72 jam proses pencairan melalui proses transesterifikasi. Didapati ia menghasilkan kandungan asid lemak bebas metil ester tak tepu rantaian C16 hingga C18. Walaubagaimanapun apabila transesterifikasi dijalankan ke atas bahan model yang terdiri daripada C2-C5, keputusan berlawanan diperolehi. Keputusan menunjukkan asid lemak bebas metil ester tepu merupakan bahan hasil utama. Oleh itu, pencairan semulajadi bahan sisa makanan mempunyai potensi untuk dijadikan bahan pembuatan biodiesel.

Kata kunci: sisa buangan makanan, asid lemak bebas, pengekstrakan, biodiesel, esterifikasi.

ACKNOWLEDGMENT

First and foremost, I would like to express my respect and gratefulness to Allah, for His blessing throughout my effort of completing this dissertation. This dissertation project had given a very good and useful lesson, not only in environmental field but also in life. It is also a very meaningful experience to work with different kind of people who involved in my project.

I would like to express my sincere gratitude to both of my project supervisors, Dr. Ghufran Redzwan and Dr. Zul Ilham Zulkiflee Lubes for their guidance throughout this project. The guidance given is highly appreciated.

My deepest appreciation goes to my beloved parents, my family and my children, Muhammad Irfan and Muhammad Irsyad for their endless support and encouragement.

Deepest thanks to all staff at Institute Science Biology (ISB) and Faculty of Science for the kindly help. The thanks dedicated to Prof. Suffian Annuar, Dr Ahmad Mohammed Gumel, Cik Norizan, Puan Zuraidah, Encik Johari and Encik Roslan.

Last but not least, thank you to all my friends and those who have assisted directly or indirectly to make this project into success.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

CHAPTER 2: LITERATURE REVIEW

CHAPTER 3: MATERIALS AND METHODS

CHAPTER 4: RESULTS AND DISCUSSION

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

COD – Chemical oxygen demand

FAME – Fatty acid methyl ester

FFA – Free fatty acids

GC-FID – Gas chromatography-flame ionization detection

GCMS – Gas chromatography-mass spectrometry

KOH – Potassium hydroxide

LCFAs – Long chain fatty acids

LCMS – Liquid chromatography-mass spectrometry

MS – Mass spectrometer

MSW – Municipal Solid Waste

MUFAs – Monounsaturated fatty acids

OA – Organic acid

OFMSW – Organic fraction of municipal solid waste

SW – Solid waste

TIC – Total ion chromatogram

TG – Triglycerides

TS – Total solid

TVS – Total volatile solid

PUFAs – Polyunsaturated fatty acids

VFA – Volatile fatty acid

VOCs – Volatile organic compound

CHAPTER 1: INTRODUCTION

1.1 General Introduction

The global crisis of fossil fuel depletion and environmental degradation has led to the exploration of a new and sustainable diesel fuel alternative. Biodiesel has become attractive as an alternative fuel for diesel engines due to its environmental benefits and renewable resources. For years, focus on feedstock for biodiesel production is mostly from edible oils, very few is concentrating on non-edible oil. Due to the fact that non-edible oil could also be used as a cost effective feedstock, the possibility of utilizing the abundance of daily food waste in this country as an alternative feedstock to replace the conventional feedstock of vegetable and animal fats is interesting to be studied. It is also believed that the use of food waste as a source for biodiesel will change the usual method of food waste treatment, which currently uses anaerobic digestion (Kondusamy et al., 2014).

In 2005, Malaysia has generated 7.34 million tonnes of municipal solid waste (MSW) and is expected to increase to 10.9million tonnes in 2020 (Abdul Hamid et al., 2012). In average, Malaysian generates 1.2 kg / day / person of solid waste (Budhiarta et al., 2012). The solid waste management in Malaysia has been considered primitive until the year of 2007, when government introduced comprehensive solid waste management and Public Cleaning Act (Yahya & Larsen, 2008). Implementation of the act by establishing waste to wealth solution, together with the support from public is seen as a way to find the best solution to overcome the issues in waste management system.

Since solid waste can be recycled into new product, exploration of sustainable biodiesel resources as alternative fuels can best replace the dependency to petroleum-based fuels. By replacing the use of vegetable oil, used cooking oil and animal fats to organic waste materials as biodiesel feedstock, it could lead to the new approach in renewable energy production. As reported by Abdul Hamid et al. (2012), 60% of the solid waste produced in this country was contributed by food waste, therefore utilizing this unlimited resources for synthesis of biodiesel is a promising way on better handling of domestic waste.

Through the process of esterification, the abundance of fatty acids typically free fatty acid (FFA) in the food waste has great potential to be used as feedstock to be converted to fatty acid methyl ester (FAME) or biodiesel. The application of food waste as a feedstock could be possible, since feedstock generated from waste cooking oil for biodiesel production has been considered successful (Talebian-Kiakalaieh et al., 2013). Although used cooking oil will mainly produce low quality biodiesel, developing the process that can utilize food waste could result to an economical process. The success of this approach is also believed could replace anaerobic digestion, which known as the most developed method in food waste treatment, other than landfill and composting (MONSAL, 2011).

Converting biodegradable organic materials typically lipids through anaerobic digestion are attractive for the biogas production due to the fact it reduced the organic materials and theoretically have high methane potential (Fernandez et al., 2005; Subramani & Ponkumar, 2012; Li et al., 2011). One of the successful story of anaerobic digestion process for food waste treatment was applied in actual processing plant by SYSAV Biotech AB in Sweden since 2009 (SYSAV, 2012). However, anaerobic treatment with high lipid content creates several problems such as sludge flotation and washout.

High lipid content in anaerobic treatment mainly consisted of long chain fatty acids (LCFAs). It has been reported that this high LCFAs concentration produced could destabilise anaerobic digester due to inhibition of methanogenic bacteria by possible damage to cellular membrane (Fernandez et al., 2005). At this phase, longer chain of FFA starts to accumulate and the balance between non-methanogen and methanogen would be interrupted and conversion of soluble organic matter to gaseous form shall end (Redzwan & Banks, 2010).

Although production of methane gas is one of the potential energy, however this type of energy has negative impact to the environment. Therefore utilizing the production of volatile fatty acid (VFA) in the biochemical cycle during the methanogenesis phase as hydrocarbon source for biodiesel production could give a better alternative for energy resources.

1.2 Aims and Objectives

It is known that esterification of FFA could form FAME and finally be used as biodiesel. This study explore typical types of FFA including volatile fatty acid (VFA) which are produced during hydrolysis and acidification of food waste in the absence of oxygen.

In order to make all types of FFA suitable as feedstock transport biodiesel, the substrates need to be converted to esters through hydrogenation. Food waste is known as waste with abundance of organic compound. FFA is one of the organic compounds that can be extracted from food waste at early stage of food waste decomposition. Currently food waste is managed through anaerobic digestion for the generation of biogas. The introduction of a naturally induced decomposition of food waste via hydrolysis or liquefaction could provide a cheaper solution, with reference to source of biodiesel feedstock. Hence, the collected quantitative data from this study is aimed to contribute to find other sustainable feedstock for biodiesel production and to provide alternative in handling food waste.

Objectives of this study are as follows:

1. To select a suitable method for fatty acids extraction.
2. To determine the composition of fatty acids from food waste as potential biodiesel feedstock.
3. To compare the composition of FFA from different stages of food waste liquefaction.
4. To verify the viability of biodiesel feedstock via esterification process.

1.3 Scope of Work

This study explores the alternative solution in the management of domestic waste and the possibility of using food waste as biodiesel feedstock. As food waste normally ended in the landfill or composting site, this study aims to provide an alternative to the usual way of handling food waste. The focal in this study is on the identification of typical types of FFA including VFA which are generated during hydrolysis and acidification of food waste in the absence of oxygen.

Therefore, the first part of the study is to evaluate the feasibility of FFA as feedstock for biodiesel production. Prior to this, method of extracting FFA from food sample was established. Comparison between the dry and wet extraction method would be carried out. For dry-extraction, moisture was removed before it was extracted for composition analysis by Soxhlet method. On the other hand, for wet-extraction method, sample was directly applied to the Reflux method.

Esterification was carried out on the substrate which was acquired from the self-hydrolysed food waste sample and also on modelled compound of selected FFA, to represent the source of substrate. As a comparison, anaerobic condition was used to determine the presence of FFA. In anaerobic condition it was studied with the addition of sludge at the stage when the production of biogas had stopped. The sludge acted as catalyst that induced the process towards the production of biogas. At this point production of organic acid was diverted to produce precursor for biodiesel synthesis.

1.4 Thesis Structure

This thesis is presented in five Chapters. Chapter 1 introduces the aims and objectives of the study. The scope of work is also mentioned in this chapter. Chapter 2 introduces the background of food waste generation and treatment associated. The main barriers to the implementation of food waste treatment technology which associated with the material, cost effectiveness of the technology are addressed and discussed in this chapter. The main concerned of energy production associated with the waste are also reviewed in this chapter.

Chapter 3 described the methodology of the extraction and purification of FFA from food waste using conventional and developed method of extraction. Comparison of FFA profile from hydrolysed food waste and modeled compound are also discussed. The potential of using the FFA as feedstock for biodiesel is also highlighted.

Chapter 4 presents the main and comparative results of this thesis. The results of two extraction technique are represented and their implication and effectiveness are discussed in detail. The Chapter also discussed the feasibility of FFA extraction at early liquefaction stage of hydrolysed sample. Verification results of this FFA and model compound as feedstock for esterification process is presented in percentage of FAME content. As comparison, the FFA production from anaerobic digestion process and the relationship between the FFA production and impact of substrate nature is evaluated.

Finally, Chapter 5 presents the conclusion of the findings of this research and recommendations for future work to improve the production of biodiesel from food waste feedstock.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Food waste, an organic fraction of municipal solid waste (OFMSW) is biodegradable with moisture content around 85-90%. In recent years, anaerobic digestion process together with this OFMSW has been used in production of methane and energy. Air emissions of anaerobic plant include certain percentage of produced methane. Since it is impossible to collect 100% of the produced biogas, certain amount ends in the atmosphere and contributed to the increasing of carbon footprint. Utilisation of significant amount of fatty acid from food waste could be a good attempt to generate biodiesel as a green product. Industrial production of biodiesel from food waste can contribute to resolve the waste disposal and energy shortage problem.

In this chapter, the findings from other literatures that related to the studied are reviewed. The primary attention is given to the findings obtained on food waste treatment technology and non-renewable resources in biodiesel production. Other aspects of biodiesel such as method, physical properties and analysis are also addressed in this part.

2.2 Food Waste

Among the proportion of organic fraction of municipal solid waste (OFMSW), the food residue is the most given attention in developing countries. Generally food waste is defined as uneaten portion of meals, leftover and trimmings from food preparation of restaurant, kitchen and cafeterias (Chua et al., 2008). Food waste or sometimes known as kitchen waste is characterized by high organic content, most of which is composed of easily biodegradable compounds such as carbohydrates, proteins and smaller lipid molecules (Gill et al., 2014).

According to Johari et al. (2012), global municipal solid waste generated in 1997 was about 0.49 billion tonnes with an estimated annual growth rate of 3.2-4.5% in developed nations and 2-3% in developing nations. It is also mentioned Peninsular Malaysia generates about 17,000 tonnes of municipal solid waste (MSW) per day where 6,200,000 tonnes/year was recorded in 2002. Related to the same report, in 2010 the estimation showed Selangor was the top MSW generator with estimation of 1,595,000 tonnes/year followed by Johor with 1,395,000 tonnes/year and thirdly Kuala Lumpur with estimate value of 1,202,000 tonnes/year

From the total MSW, food waste or organic waste in Malaysia is made up 40-45% of the characterization study of solid waste (SW) composition , followed by plastic and mix paper as shown in Table 2.1 (Kalanatarifard et al., 2012; Johari et al., 2012). In most cases, the main constituents of waste generated are similar throughout the world, however the quantity generated, the density and the proportion of streams vary extensively between regions. This was influenced by many factors such as level of economic development, urbanization level, lifestyle, cultural norms, geographical location, energy sources and weather condition (Syed Ismail & Abd Manaf, 2013; Agamuthu, 2001).

Table 2.1: Solid waste composition in Malaysia ([a]Kalanatarifard et al., 2012; [b]Johari et al., 2012).

Type of Waste	**Average (%)**
Organic waste[a,b]	42-45
Paper[a,b]	7-10
Glass[a,b]	3
Metal[a,b]	6
Others[a,b]	9-15

Study done in Kuala Lumpur reported, food waste and its mixtures contributed 0.6 kg / capita / day of daily SW production (Budhiarta et al., 2012). Laboratory data reported by Abdul Hamid et al. (2012) on food waste composition from household in Malaysia indicated food waste composition generally consists of moisture, ash, total sugar, carbohydrate, protein, fats and fiber. However this data does not represent the Malaysian food waste as there is no actual study on food waste composition collected from household or disposed at landfill. Another report stated that Malaysian food waste consists of rice (60%), followed by fish and meat (20%) and vegetable and fruit (20%) (Hafid et al., 2010).

Food waste is characterized by pH, chemical oxygen demand (COD), total solid (TS), total volatile solid (TVS) and moisture content which the value could be vary from country to country (Vikrant & Shektar, 2013). As food waste consists of highly biodegradable organic content, it caused leachate, odour, methane and high water content. Agamuthu (2001) stated the moisture content of MSW in developing countries is very high. The author reported MSW in Malaysia has a moisture content of about 75%. The value reported is slightly high but not in contradict to the study conducted by Johari et al. (2012). In his study on simulated and actual waste, he showed that MSW contain 50-65% of moisture content. High moisture content particularly in kitchen waste is one of the potential problems if energy is derived in the form of steam, where huge amount of fuel would be wasted in drying process (Apte et al., 2013).

Table 2.2: Analysis of simulated and actual MSW ([a]Agamuthu, 2001; [b]Johari et al., 2012; [c]Rozainee et al., 2008).

Analysis	**MSW[a]**	**MSW[b]**	**MSW[c]**	**Simulated**
Moisture Content (%)	75.00	55.01	61.71	52.34

In term of composition, food waste composition varies from animals to vegetables fraction. Due to this reason, it causes the production of organic acids (OA) in acidogenesis stages. Hafid et al. (2010) has developed a model kitchen waste to overcome the variation of kitchen waste composition in the fermentation process. The results indicate OA concentration in model kitchen waste was slightly lower compared to kitchen waste. High soluble organics content in food waste also make them rapidly converted to volatile fatty acid (VFA) at early stage of digestion (Cho et al., 1995).

One of the major organic matters found in food waste is lipid which is characterized either as fats or oils and greases. Lipids in food waste mainly consist of triacylglycerides and long chain fatty acids (LCFAs). These lipids are reduced organic materials and have high theoretical methane potential (Fernández et al., 2005).

2.3 Fats and Oils

Fats and oils are primarily non water-soluble, hydrophobic substances in the plant and animal that is made up of one mole of glycerol and three moles of fatty acids. It is commonly referred as triglycerides (Singh & Singh, 2010). Food waste leachate which is a sludge-like mixture contains various oil components accounting for 5g/L. Leachate is mostly compost of vegetable or animal fats and oils (Kim et al., 2011).

It is reported the net calorific values of fats and oils are ranging between 36.25-37.30 J/g. This is 6-8% less than the gross calorific value (Gravalos et al., 2008). Fatty acids esterified to glycerol, are the main constituents of oils and fats (Scrimgeour, 2005).

The individual characteristic of the chain generally influence the calorific value and viscosity of the biodiesel produced. As the chain length increase, the calorific value of biodiesel also increases therefore give a high viscosity (Cao et al., 2014). Fatty acids content in some of the biodiesel feedstock are listed in Table 2.3.

Table 2.3: Fatty acids characterization of biodiesel feedstock

Fatty acids	**Hazelnut oil** (Bada et al., 2004)	**Mustard oil** (Alam et al., 2013)	***Jatropha curcas L.*** **(Argentinian seed)** (Montes et al., 2011)	***Jatropha curcas L.*** **(Paraguayan seed)** (Montes et al., 2011)	**Waste cooking oil** (Talebian-Kiakalaieh et al., 2013)
C16:0	5.43	1.74	10.0	13.2	11.8
C16:1	0.23	0.17	-	-	-
C18:0	2.84	1.04	5.4	3.0	4.4
C18:1	83.47	9.56	30.2	40.2	25.3
C18:2	10.62	13.31	53.3	42.6	49.5
C18:3	0.15	11.10	-	-	-
C20:0	0.15	6.36	-	-	-
C20:1	0.17	1.65	-	-	-
C22:0	0.20	42.16	-	-	

2.3.1 Fatty acids

Fatty acids compound that contributed to the structure of fats are almost entirely straight chain aliphatic carboxylic acids. The most common natural fatty acids are C4 to C22 with C18 is the most common. The chain of fatty acid that contains double bond is known as unsaturated and without double bond is known as saturated (Misra et al., 2010).

These natural fatty acids normally have a common biosynthesis. The chain is built from two unit carbon units, and *cis* double bonds are inserted by desaturase enzymes at specific positions relative to the carboxyl group (Scrimgeour, 2005). Fatty acids such as long-chain polyunsaturated fatty acids (PUFAs) are used to characterize the quality of oils (Bernal et al., 2013). Table 2.4 shows the common name and structure of common fatty acids.

Table 2.4: Common name and structure of fatty acids (Singh & Singh, 2010)

Name of fatty acids	**Common name**	**Structure (xx:y)**	**Formula**
Lauric	Dodecanoic	12:0	$C_{12}H_{24}O_2$
Myristic	Tetradecanoic	14:0	$C_{14}H_{28}O_2$
Palmitic	Hexadecanoic	16:0	$C_{16}H_{32}O_2$
Stearic	Octadecanoic	18:0	$C_{18}H_{36}O_2$
Oleic cis-9	Octadecanoic	18:1	$C_{18}H_{34}O_2$
Linoleic cis-9,cis-12-	Octadecadienoic	18:2	$C_{18}H_{32}O_2$
Linolenic	cis-9,cis-15,cis-15-Octadecatrienoic	18:3	$C_{18}H_{30}O_2$
Arachidic	Eicosanoic	20:0	$C_{20}H_{40}O_2$
Behenic	Docosanoic	22:0	$C_{22}H_{44}O_2$
Erucle	cis-13-Docosenoic	21:0	$C_{32}H_{42}O_2$
Lignoceric	Tetracosanoic	24:0	$C_{24}H_{48}O_2$

In biodiesel, the amount of unsaturated and saturated fatty acids play significant role in determining the inbuilt oxidative stability (Fakhry & Maghraby, 2013). Monounsaturated fatty acids (MUFAs) are the best components for biodiesel when considering the oxidative stability and low temperature fluidity (Cao et al., 2014). According to Chhetri et al. (2008), soapnut *Sapindus mukorossi* oil and *Jatropha curcas* oil produced 97% conversion to FAME.

Different type of fatty acids chain in biodiesel generally involved different area of study. Production of biodiesel involving the MUFAs mainly are from microalgae feedstock where the focus is on the metabolic synthesis compared to LCFAs where the focus is mainly on energy production. A model study on MUFAs and LCFAs, found that model microorganism of *Escherichia coli (E.coli)* produced free MUFAs which are palmitoleate and cis-vaccenate (Cao et al., 2014).

LCFAs represent an important fraction of the organic matter in wastewater. In one of the study associated with LCFAs, the model observation indicated fermentation in low concentration of fatty acids is non-spontaneous, however in the high concentration it may be better digested if facilitated by the provision of supplemental thermal energy (Oh & Martin, 2010).

2.3.2 Free fatty acid

The use of edible oils as feedstock for biodiesel synthesis will compete with food supply and relatively costly. As an alternative, feedstock from inedible oils, animal fats, waste food oil and by-product of the refining vegetable oils have the potential to lower the production cost of biodiesel. However this feedstock contain significant high amount of free fatty acid (FFA) that make them inadequate for direct base catalyzed transesterification reaction (Kombe et al., 2013;Yu et al., 2011). It is stated waste cooking oils and animal fats contain significant amount of FFA. This FFA appear as a form of used vegetable oils, yellow grease (8-12wt % FFA), brown grease (>35 wt % FFA) and soapsoacks (by product of refining vegetable oils).

FFA contents are one of the most frequently determined quality indices during edible oils production, storage and marketing. It is a measurement of the extent hydrolysis which has liberated fatty acids from their ester linkage with parent triglyceride molecule (Mohamed et al., 2013).

In biodiesel production, total FFA content related with the feedstock must not exceed 0.5 wt% in case of base catalyzed process since it will produced lower fuel grade biodiesel (Dholakiya, 2012).

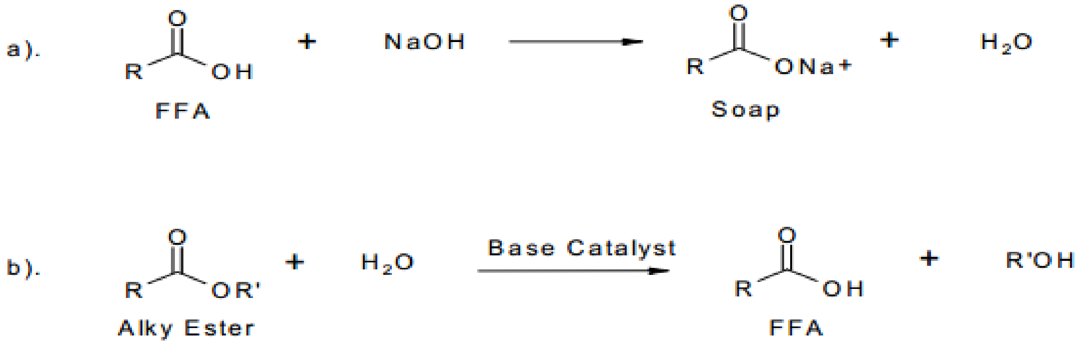


Figure 2.1: (a) Base catalyst reaction with FFA to produce soap and water.
(b) Water promotes the formation of FFA (Dholakiya, 2012).

The titration results of FFA content of base transesterification found that FFA content of crude palm kernel oil is 7.23% (Viele et al., 2013). The result obtained is higher than the same study done by Babalola & Apata (2011) where they found FFA content of palm kernel oil is 7.05%. In the same study, they also listed the chemical FFA content of some alternative lipid sources (Table 2.5).

Table 2.5: FFA content of different lipid sources (Babalola & Apata, 2011)

Sources	**FFA (% of oleic acid)**
Palm kernel oil	7.05
Soybean oil	1.54
Palm oil	3.55
Sunflower oil	4.82
Coconut oil	7.33
Groundnut oil	5.26
Melon seed oil	2.05

Study done on esterification pretreatment of FFA shows FFA conversion under different methanol usage is influenced by the reaction temperature. It is reported the higher the reaction temperature, the more complete the FFA conversion would be. The optimal reaction temperature range reported was 55 ^{o}C-65 ^{o}C (Chai et al., 2014).

In a kinetics study of *Cucurbitapepo L.* seed oil for biodiesel production, it was reported that optimal rate for methylation and butylation was at 6.1 alcohol/oil ratio, with stirring rate of 200 and 400 rpm and 3% by weight of catalyst (H_2SO_4) (Ogbu & Ajiwe, 2013).

2.4 Waste to Energy

Energy from waste is a concept that has been applied for years. In Malaysia, such thermal treatment, recovery or waste to energy was introduced as new treatment methods (Sreenivasan et al., 2005). The policy of municipal solid waste management in Malaysia was quite primitive until the late 1970s.

The situation continued until government introduced the Solid Waste and Public Cleansing Management Act 2007 (SWPCM Act 2007) in 2007. The main strategies in this Act are to implement efficient solid waste treatment, interim treatment and final disposal of solid waste (Abdul Jalil, 2010).

Report by Abdul Jalil (2010) stated urban population which constitutes more than 65% of the total population in Malaysia is generating more organic waste than the rural population. Due to this nature, varieties of waste disposal treatment method have been introduced. Impact of technology both in energy and raw materials were given consideration before the waste disposal treatment is introduced. Dhokhikah et al. (2012) stated methods for final SW treatment and disposal in developing Southeast Asian countries were commonly open dumping (more than 50%), landfill (10-30%), incineration (2-5%) and composting (less than 15%). In one similar report, Abdul Jalil (2010) reported Malaysia produced 70% of SW for final disposal with 20-30% is dumped or thrown into river.

As the waste management hierarchy continues its evolutions, consumption of landfill sites are nearly exhausted and newly landfill sites are hardly available due to several reasons. Among the reasons are shortage of land and lack of public acceptance (Emmanuel et al., 2007). In addition, uncontrolled fermentation of organic wastes in landfill causes emission of greenhouse gasses such as methane and carbon dioxide. Leachate in food waste also caused groundwater pollution which required further treatment (Moukamnerd et al., 2013). Meanwhile incineration has been criticized due to high cost and impact to the environment (Hashim et al., 2012).

There are different methods of waste treatment methods practiced in Malaysia as shown in Table 2.6. In general, solid waste is managed for the following purposes; recycling solid waste, reuse of waste and composting. The preferred method of waste management is using technology through landfill where the most sites are open dumping.

Disposal of MSW through landfilling is preferable due to certain factors such as financial, social and technical (Shamsudin et al., 2013). However, treatment technique for MSW very much depends on the heavy metals content which commonly is Cadmium, Nickel, Zinc, Copper, Lead, Mercury and Chromium. Study done by Rashad & Shalaby (2007) showed highly polluted heavy metals in two dumpsites with different distance in Alexandria, Egypt which confirmed the content of these heavy metals. If sanitary landfilling is used, proper precautions need to be taken so that these heavy metals do not enter the underground water through the leachate (Agamuthu, 2001). Leachate is produced by reaction between water percolating through the landfilling and waste (Ghosh et al., 2013).

Table 2.6: Waste treatment methods practiced in Malaysia (Samsudin & Don, 2013)

Treatment Methods	**Year 2002 (%)**	**Year 2006 (%)**	**Target 2020 (%)**
Recycling	5.0	5.5	22.0
Composting	0	1.0	8.0
Incineration	0	0	16.8
Inert landfill	0	3.2	9.1
Sanitary landfill	5.0	30.9	44.1
Other disposal sites	90.0	59.4	0
Total	100.0	100.0	100.0

Among waste treatment practiced, composting is technology widely used for the treatment of solid organic wastes. Composting is treatment process where it allows the wastes to be disposed of by reducing their size and volume (Yadav et al., 2013; Pagans et al., 2006). Volatile organic compounds (VOCs) are among the major pollutants found during composting. Pagans et al. (2006) treated the exhaust gas generated from composting with biofilter and found that composition of VOCs mixture obtained during the composting of organic waste has dramatic effect on the performance of biofilter. It also noticed efficiencies in the biofiltration of exhaust gases from animal by-product composting were lower than 30% due to the lack acclimation of microorganisms.

One of the popular bio-technique for converting the solid organic waste into compost is earthworm farming or known as vermitechnology. According to Aalok et al. (2008) vermicomposting facilities have been developed in domestic and industrial marketing in countries such as Canada, United States of America, Italy and Japan. Generally vermitechnology consists of three main processes;

- Vermiculture – rearing of earthworms .
- Vermicomposting – biodegradation of waste biomass in earthwormic way.
- Vermiconvertion – mass maintenance of sustainability of waste lands through earthworms.

Study done on vermicomposting using earthworms (*Eisenia Fetida*) produced from food waste on chemical parameter (pH, carbon to nitrogen contents (C/N)) and germination bioassay) shows vermicompost has stable value of C/N ratio. However it is stated stability test alone was not able to ensure high vermicompost (Majlessi et al., 2012).

Even though composting technology of MSW is well established, only a few of the refuse composting plant around the world are economically successful. The obstacle commonly experienced with composting is the high cost and low value of the compost products (Sreenivasan et al., 2012).

Due to these factors, waste technology concept based on physicochemical properties of the waste, type and quantity of waste feedstock and desired form of energy has been introduced. Generally, conversion of solid waste to energy is undertaken using three main process technologies. There are thermochemical, biochemical and mechanical extraction. Anaerobic digestion and fermentation are example of biochemical technology. While combustion, gasification, pyrolysis and liquefaction are processes include under thermochemical conversion. As for mechanical extraction, oil production from seeds of solid waste product is example of process (Eddine & Salah, 2012).

In a study by Hesnawi & Mohamed (2013), it is reported food waste has potential for methane production. The authors stated potential of methane production is depending on the type of food used with ranging from 96 to 426 ml dry g^{-1}. It also mentioned that methane yield on different food waste, cooked meat, boiled rice, fresh cabbage and mixed food waste were 482, 294, 277 and 472 ml/g volatile solid (VS) at 37 ^{o}C.

Rao & Singh (2004) studied the bioenergy conversion of organic fraction of municipal solid waste (OFMSW). The authors reported, bioenergy yield from municipal garbage and corresponding bioprocess conversion efficiency over the length of the digestion time were observed to be 12,528 kJ/kg volatile solids and 84.51% respectively.

In order to optimize the waste to energy concept, challenges arising through the application of the concept need to be given special attention. Among the challenges are lack of versatility, waste-gas cleanup, conversion efficiency, regulatory hurdles and high capital costs (Eddine & Salah, 2012).

2.5 Bio-energy

The demand of energy for transportation, heating and industrial processing is increasing daily. Energy production from renewable sources has been given priority since it provides energy sources that protect the environment. In recent years, bio-energy sources have become more important as viable and economical alternative sources (Duhan et al., 2013). In general, bio-energy is the chemical energy contained in organic materials that can be converted into direct, useful energy sources via biological (including digestion of food), mechanical or thermochemical processing (Amthor & Tan, 2013).

In Malaysia, developments of bio-energy from alternative resources playan important role where government has implemented several policies and strategies towards green technology on energy sector. The aimed is towards the environmental protection. The starting was Four Fuel Diversification Strategy in 1980, where the focused was on development of hydrocarbon and the use of natural gas and coal.

In 8th Malaysia Plan (2001-2005), the fifth fuel strategy was introduced to promote the use of renewable energy as a solution in addressing the issue on climate change. Continuing initiative by the Malaysian government to promote renewable energy is enhancing in the use of renewable energy and biomass in 9th Malaysia Plan (2006-2010) (Hashim et al., 2012).The launched of The National Biofuel Policy in March 2006 proposed the production of B5 blend biodiesel (comprising 5% processed palm oil with 95% petroleum diesel) for the local market. It was estimated B5 composition would produce 500,000 tonnes with additional demand for palm oil, contributing 30% of 2006-2009 average palm oil stock (Jaafar et al., 2011). The plan has shown achievement in the use of environmental friendly, sustainable and viable sources of biomass energy. The target which set under Five Fuel Policy stated biomass was identified as one of the potential renewable energy.

To ensure effort on the environment sustainability continues various measures has been implemented in 10th Malaysia Plan (2011-2015). Introduction of the Feed-In Tariff and Renewable Energy Fund showed government serious commitment in encouraging more implementation of renewable energy projects. The National Biofuel Policy that was launched in March 2006, emphasized on the research and development of biofuels in order to reduce the dependency on fossil fuels.

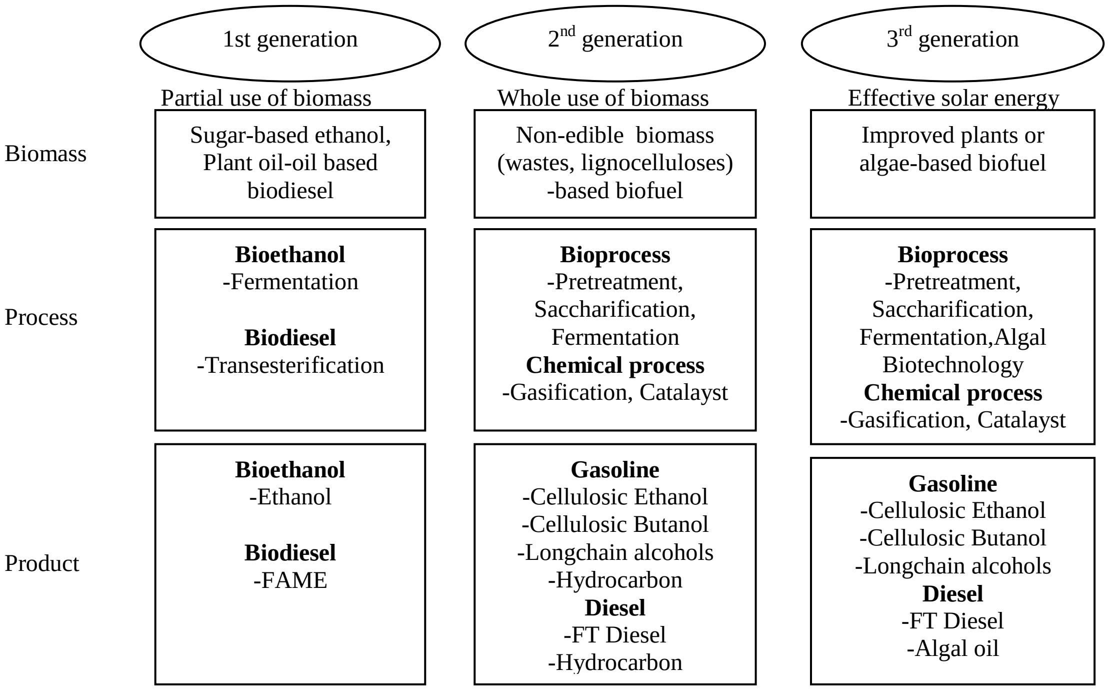
In general bio-energyincludes bioethanol, biobutanol, biodiesel, biomethanol, pyrolysis oil, biogas and biohydrogen (Saleh & Rajanaidu, 2011).

2.5.1 Biofuels

Biofuels are referring to the fuels for direct combustion of electricity production. It is mainly used for liquid fuels in transportation sector. Biofuels are predominantly produced from biomass resources (Sahu, 2014; Gnanaprakasam et al., 2013).

First-generation of biofuels derived from sugar, starch, vegetable oil or animal fats using conventional technology (Chang et al., 2010). Expansion of biofuels from first-generation, gives a variety of concerns such as the increase in food price, implication to the poor, expansion of agricultural land, impact on natural habitat and increase in use of agrichemical (World Bank, 2007).

Due to these factors, attention has been given in recent years to the second-generation of biofuels which the sources come from non-food biomass (Sims et al., 2010). These include waste biomass, the stalks of corn, grass, wood and special energy or known as biomass crop (Chang et al., 2010). Figure 2.2 illustrates the three generation of biofuels and its related process.



	1st generation Partial use of biomass	2nd generation Whole use of biomass	3rd generation Effective solar energy
Biomass	Sugar-based ethanol, Plant oil-oil based biodiesel	Non-edible biomass (wastes, lignocelluloses) -based biofuel	Improved plants or algae-based biofuel
Process	**Bioethanol** -Fermentation **Biodiesel** -Transesterification	**Bioprocess** -Pretreatment, Saccharification, Fermentation **Chemical process** -Gasification, Catalayst	**Bioprocess** -Pretreatment, Saccharification, Fermentation,Algal Biotechnology **Chemical process** -Gasification, Catalayst
Product	**Bioethanol** -Ethanol **Biodiesel** -FAME	**Gasoline** -Cellulosic Ethanol -Cellulosic Butanol -Longchain alcohols -Hydrocarbon **Diesel** -FT Diesel -Hydrocarbon	**Gasoline** -Cellulosic Ethanol -Cellulosic Butanol -Longchain alcohols **Diesel** -FT Diesel -Algal oil

Figure 2.2: Generation of biofuels (Chang et al., 2010)

Wastes from the food industry can be used as energy sources as well as any other carbohydrates. Stoeberl et al. (2011) mentioned fermentation of biobutanol from waste-whey is suitable for fermentative production of biofuels.

Table 2.7 shows the comparison of fuel properties on diesel, rapeseed oil, butanol, methanol and ethanol. It is shown diesel contain high number of cetane, followed by rapeseed oil. In relation to fuel, cetane number plays an important parameter with the ignition delay. As cetane number increase, the temperature in the combustion chamber increase. Formation of particulate matter will increase the oxidation rate and reduces the emission of unburned hydrocarbon (Cataluna & Silva, 2012).

Table 2.7: Comparison of fuel properties (Stoeberl et al., 2011)

	Unit	**Diesel**	**Rapeseed oil**	**n-butanol**	**Methanol**	**Ethanol**
Cetane number	-	44-55	50	12	3	8
Density	g/ml	0.86	0.92	0.81	0.8	0.79
Auto-ignition temperature	^{0}C	200-220	>300	385	470	434
Lower heating value	MJ/kg	42.5	37.6	35.1	19.9	26.8
Boiling point	^{0}C	180-230	>350	117	64.5	78.4
Saturation temperature	^{0}C	-	-	20	20	20
Saturation pressure	kPa	-	<0.1	0.6	11.83	5.93

2.5.2 Biogas

Biogas is a flammable gas consists of mainly methane and carbon (IV) oxide with traces of hydrogen sulphide and water vapour that produced when organic materials are fermented under anaerobic condition. The outcome of anaerobic digestion process of food waste at large scale to produce biogas is more highlighted compared to landfill and composting (MONSAL, 2011). As organic waste is the highest portion in Malaysian MSW composition, the concern on the waste management is focusing on this organic waste particularly food waste. It is reported, methane generation has been applied to meeting the energy needs in country such as England, India and Taiwan. In those countries methane generating units as well as plants using cow manure and municipal waste has been operated for years (Vikrant & Shekhar, 2013).

Anaerobic digestion is one of the most developed methods that emphasized in reducing waste volume, waste stabilization and biogas recovery (Vintila et al., 2013; Amankwah et al., 2012; Vikrant et al., 2013). In anaerobic digestion process, biogas is produced by natural process involving the decomposition of organic material under anaerobic conditions (Bagudo et al., 2011; Subramani & Ponkumar, 2012; Li et al., 2011). Four metabolic pathways involved in overall anaerobic digestion; Hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis as shown in Figure 2.3.

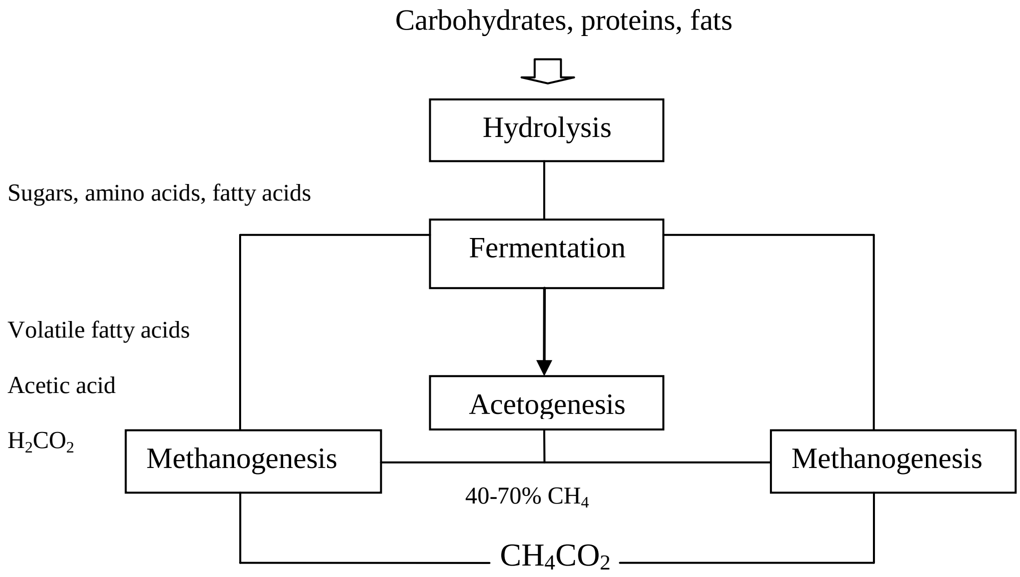


Figure 2.3: Metabolic stages of anaerobic digestion (Li et al., 2011)

In acidogenesis phase, pH plays a significant role because some acid producing bacteria prefer pH ranging from 5.5 to 6.5 to produce acid. Hafid et al. (2010) reported the highest level of organic acid produced in acidogenesis phase was 77g/L at optimum pH (6.02), temperature (35-37 ^{o}C) and inoculum size (20%). pH control in acidogenic anaerobic fermentation also shows improvement in VFA production in anaerobic reactors treating organic solid waste (Bolzonelle et al., 2005). It is also reported, implementation of this technology to agricultural wastes, food wastes, and wastewater sludge has proven successful due to its potential of reducing chemical oxygen demand (COD) and biological oxygen demand (BOD) from waste streams and producing renewable energy (Li et al., 2011).

The process reached the onset of methanogenic phase at day 63 and the methane production was greater at a moisture level of 70% (Khalid et al., 2011). It is also stated, production of larger volatile fatty acids from such waste inhibits the activity of methanogenic bacteria. Although anaerobic digestion process is environmentally valuable technology, it has some disadvantages such as long retention time and low removal efficiencies of organic compound (Kim et al., 2003).

Amount of biogas produced from the digestion process depends on several parameter such as pH, temperature, composition of substrate, retention time and organic loading rate (Singh et al., 2014). In similar study of anaerobic biodegradation of food waste and fruit residues during biogas generation indicates the rate of gas generation decreases with sampling and residence time (Wanasolo et al., 2013).

Study done on comparison between biogas generating capacity of corn stalks bagasse found that biogas resulted from the process of lignocellulosic ethanol production produced higher yields compared to the corn stalks that are directly use as feedstock (Vintila et al., 2013).

2.5.3 Biodiesel

Biodiesel or FAME is known as biomass-derived diesel fuels which are non-aromatic and sulphur-free as compared with petrodiesel (Sani et al., 2013). The source of these triglycerides derived from virgin vegetable oils to waste cooking oil, animal fats, and soapstocks (Boucher et al., 2008). More than 350-oil bearing crops have been identified as potential sources for producing biodiesel, however only oil palm, jatropha, rapeseed, soybean, sunflower, cottonseed, safflower and peanut oils are considered as viable feedstocks for commercial production.

As biodiesel known to derive from vegetable oil and animal fat source, it is often being debated for the vegetable oil which are also the edible oil to become “Food vs. Fuel”, as both groups of oil is to be the energy source that is renewable based on its sustainability (Chai et al., 2014). As a result, the intensive research on organic waste materials as the source of biodiesel feedstock has been explored. Despite the fact that used cooking oil could produce low quality of product, Uddin et al. (2013) stated production cost is much higher for virgin vegetable oil compared to petroleum based diesel. In his study, he found that the use of waste frying oil is an effective way to reduce the raw material cost because waste frying oil is estimated to be about half the price of virgin oil.

Production of biodiesel is mainly associated with amount and type of FFA, high viscosity, low volatility and polyunsaturated characters of triglycerides (Chhetri et al., 2008; Singh & Singh, 2010). It is stated waste cooking oils and animal fats contain a significant amount of free fatty acid (FFA). This FFA appear as a form of used vegetable oils, yellow grease (8-12 wt % FFA),brown grease (>35 wt % FFA) and soapstock (by product of refining vegetable oils) which are identified as potential feedstock for biodiesel production (Singh & Singh, 2010; Hossain et al., 2010; Boucher et al., 2008; Marchetti et al, 2005; Sani et al., 2013). However, feedstock high in FFA is not easily converted by homogeneous base transesterification, because of the concurrent soap formation of the FFA with the catalyst which significantly leading to substantial yield loses (Kombe et al., 2013).

Generally, biodiesel production is obtained from four methods or processes: pyrolysis, microemulsion, dilution and transesterification (Singh & Singh, 2010). Transesterification is the most preferred method in biodiesel production (Mathiyazhagan et al., 2011; Boucher et al., 2008).

Majority of biodiesel production through this method is using application of batch reactor technology. Batch reactor technology utilized intense mixing to create and maintain a stable emulsion in order to minimize mass transfer limitations and allow the reaction to reach kinetic equilibrium (Boucher et al., 2009).

Saponification values in biodiesel production indicate the presence of fatty acids. High saponification values associated with high percentage of fatty acids in the oil. Therefore it implies the possible tendency to soap formation and difficulties in separation of product (Ibeto & Okoye, 2012).

In one related study done on saponification values, it was reported saponification value of waste cooking oil as 186.3 mg KOH/g. It also reported energy consumption for waste cooking oil transesterification; using microwave-heating method consumes less than 10% of the energy to achieve the same yield as the conventional heating method for given experimental conditions (Patil et al., 2012).

2.5.3.1 Transesterification

Transesterification (also called alcoholysis) is the reaction of a fat or oil with an alcohol to form esters and glycerol. In transesterification process, parameters such as FFA content, water and non-saponificable substances is the key to achieve a high conversion reaction. Another important variable in transesterification process is the alcohol-to-oil volume ratio where various type of alcohol (primary, secondary, straight and branched- chain) is employed. Transesterification of triglycerides (TG) of vegetable oil and methanol is most common practiced due to its favourable kinetics (Mathiyazhagan et al., 2011; Boucher et al., 2008).

The production from petroleum-based methanol is not really a genuine biodiesel. Ilham and Saka (2011) have established new biodiesel production processes comprising one-step and two-step supercritical from green reagent, dimethyl carbonate. The study indicates supercritical dimethyl carbonate does not produce glycerol and produced high yield of FAME (over 90 wt%).

In other report by Hossain et al. (2010), it stated biodiesel yield from waste canola oil increased in the order of 1-butanol <etanol< methanol with little difference in viscosity, acid value and chemical elements such as Ferum (Fe), Magnesium (Mg), Calcium (Ca), Natrium (Na) and Potassium (P) at different parameters. Although the alcohol ratio used in this study is lower than the optimal ratios suggested by most study, but the finding is very useful in term of biodiesel production cost effectiveness where significantly by decreasing the alcohol amount the higher production of biodiesel will be obtained.

In general, procedure of transesterification is based on stoichiometric reaction where 1 mol of oil is required to react with 3 mol of alcohol to obtain 3 mol of FAME (Romano & Sorichetti, 2011; Mathiyazhagan et al., 2011; Boucher et al., 2008; Yu et al., 2011). One of the main problems in biodiesel production is the reaction of saponification. Saponification (formation of soap) in vegetable oil is reported to be high and most literature stated the vegetable oil can be pretreated with acid catalyst. This acid catalyst will esterify FFAs to form esters of FFAs (biodiesel) (Ganaprakasam et al., 2013; Mishra et al., 2013; Nakpong & Wootthikanokkhan, 2010). However, acid catalyst reaction is slower than base catalyzed transesterification (Ganaprakasam et al., 2013; Mishra et al., 2013; Nakpong & Wootthikanokkhan, 2010). The transesterification method on different sample is listed in Table 2.8.

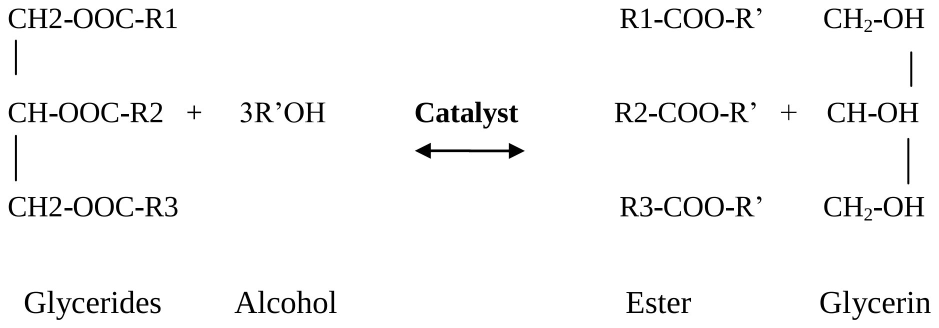
Table 2.8: Transesterification method on different sample

Sample	**Catalyst**	**Temperature**	**Alcohol to oil ratio**	**Biodiesel Yield (%)**
Waste canola oil (Hossain et al., 2010)	NaOH	60 °C	3:1	49.50
Galician marine algae (Sánchez et al., 2012)	NaOH	60 °C	6:1	17.10
Sunflower oil (Sánchezet al., 2012)	NaOH	60 °C	6:1	91.97
Azadirachtaindica (Awolu et al., 2013)	NaoH	50 °C	4.5:1	89.69

Two steps involved in transesterification process. The first is acid catalysed esterification where carboxylic acid is esterified by alcohol in the presence of a suitable acidic catalyst. In this step, presence of water is not preferable and it results to incomplete esterification. More often mineral acids such as acid sulfuric (H_2SO_4) besides derivate of toluene sulfonic acid is used in this step. However, corrosive action of the liquid acid catalyst and high quality of by-product is the problem linked with this application (Cardoso et al., 2008).

After the acid-catalysed esterification, the residual oil fraction then further treated under base-catalyst to produce FAME (Yu et al., 2011; Mathiyazhagan et al., 2011; Boucher et al., 2008; Cardoso et al., 2008). Base-catalysedtransesterification is widely used since the reaction time is faster than acid-catalysed (Kusdiana & Saka, 2001; Marchetti et al., 2007; Kargbo, 2010).

The systematic diagram of transesterification process is shown in Figure 2.3. Overall process is mainly a sequence of three consecutive steps which are reversible reactions. In first step, triglycerides will form diglycerides. Second step, diglycerides will produce monoglycerides and in the last step, glycerol is obtained. Esters are produced in all reactions.



$$\begin{array}{lcccccc} \text{CH2-OOC-R1} & & & & \text{R1-COO-R'} & & \text{CH}_2\text{-OH} \\ | & & & & & & | \\ \text{CH-OOC-R2} & + & \text{3R'OH} & \underset{\longleftrightarrow}{\textbf{Catalyst}} & \text{R2-COO-R'} & + & \text{CH-OH} \\ | & & & & & & | \\ \text{CH2-OOC-R3} & & & & \text{R3-COO-R'} & & \text{CH}_2\text{-OH} \\ \text{Glycerides} & & \text{Alcohol} & & \text{Ester} & & \text{Glycerin} \end{array}$$

Figure 2.4: Transesterification reaction of triglycerides and methanol to form biodiesel.

Since biodiesel production originates is originates from the mixture of ester, additional steps are necessary to obtain a product that complies with international standard (Ramano & Sorichetti, 2011).

2.6 Summary

Primary treatment of food waste very much relies on anaerobic digestion for the production of biogas. Anaerobic digestion is bounded to process stability, which generally it could not be called as sustainable resources. Alternatively, biodiesel made from the oils of various types of oil seed crops has become very attractive as a biofuel of its environmental benefits.

Study on waste materials as sources of biodiesel is one of the ways to resolve the issue of vegetable feedstock which is also a food resource. The main problems associated with feedstock from waste materials are the quality of product obtained.

Utilising FFA production to waste-energy concept will provide better alternative on how the domestic waste is currently being handled. Although study on conversion from food waste to FAME has been carried out earlier, there is still limited information on the economical quantity and quality of biodiesel.

At any stage, food waste contains abundance of organic compounds. Extraction of FFA from food waste could begin at the earliest stage of food waste decomposition. To make all type of FFA suitable for the transport fuel, the substrates need to be converted to esters through hydrogenation process. This will be the focus of the study, with particular emphasis on nature of substrate, process and FAME product.

Modelled compound is used in this study to compare the FAME production from pure substance. The validation part will be discussed in Chapter 4 and 5.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

This chapter includes detail experimental procedures and design of extrication and synthesis of biodiesel from food waste liquefaction. The design of the experimental was widely adopted to study the effect of FFA extraction on two extraction methods and early stage of liquefaction food waste as feedstock for biodiesel. Early stage refers to beginning of the stage before hydrolysis occurred.

Liquefaction of food waste without the seed sludge was carried out to investigate the production of FFA by the presence of natural microorganism. Study of the early stage liquefaction without seed sludge was carried out in three stages (24, 48 and 72 hours) under the main study. Natural liquefaction generally produces generous amount of organic acid. As addition to the main study, reintroducing microorganism from the secondary biosludge of sewage treatment plant (STP) was used as the seed sludge. This sludge was added as a catalyst for induction purpose on production of methane (CH_4).

Comparative study was done on the type of FFA profile for both liquefaction processes (with and without seed sludge). In general the study was not to improve the production of methane from food waste, but to divert the production of organic acid as the precursor of biodiesel.

3.2 Experimental Techniques

Figure 3.1 shows the experimental flow used in this study. Natural liquefaction of food waste used as the overall study. The experimental stage was carried out in three different phases; 24, 48 and 72 hours. The free fatty acid (FFA) or the oily substrate was extracted through two methods of extraction; standard method using Soxhlet and developed method using Reflux. The standard extraction method using Soxhlet required a dried sample and long extraction process. Reflux method was developed to overcome the drawbacks of Soxhlet method. The basis criterion of developing Reflux method was based on application of wet sample in the extraction method, shorter processing time and solubility of solvent in the process.

Seed sludge was added in the induced liquefaction as addition of the main study. The sludge was added to investigate the type of FFA profile during the liquefaction process, where that it could encourage higher production of FFA which can be used as the precursor of biodiesel.

Verification of type of FFA to be used as the biodiesel feedstock was done during the 72 hours natural liquefaction. Verification of similar FFA to be used as the biodiesel precursors or feedstock were modeled based on substrates consist of short chain fatty acids (C2-C5) or also known as SCFAs. Modelled substrates consists of C2-C5 compound was used in the esterification process as SCFAs in the free state or esterified to glycerol can be converted completely to methyl esters. The profiling of FAME product from esterification process was compared to biodiesel composition.

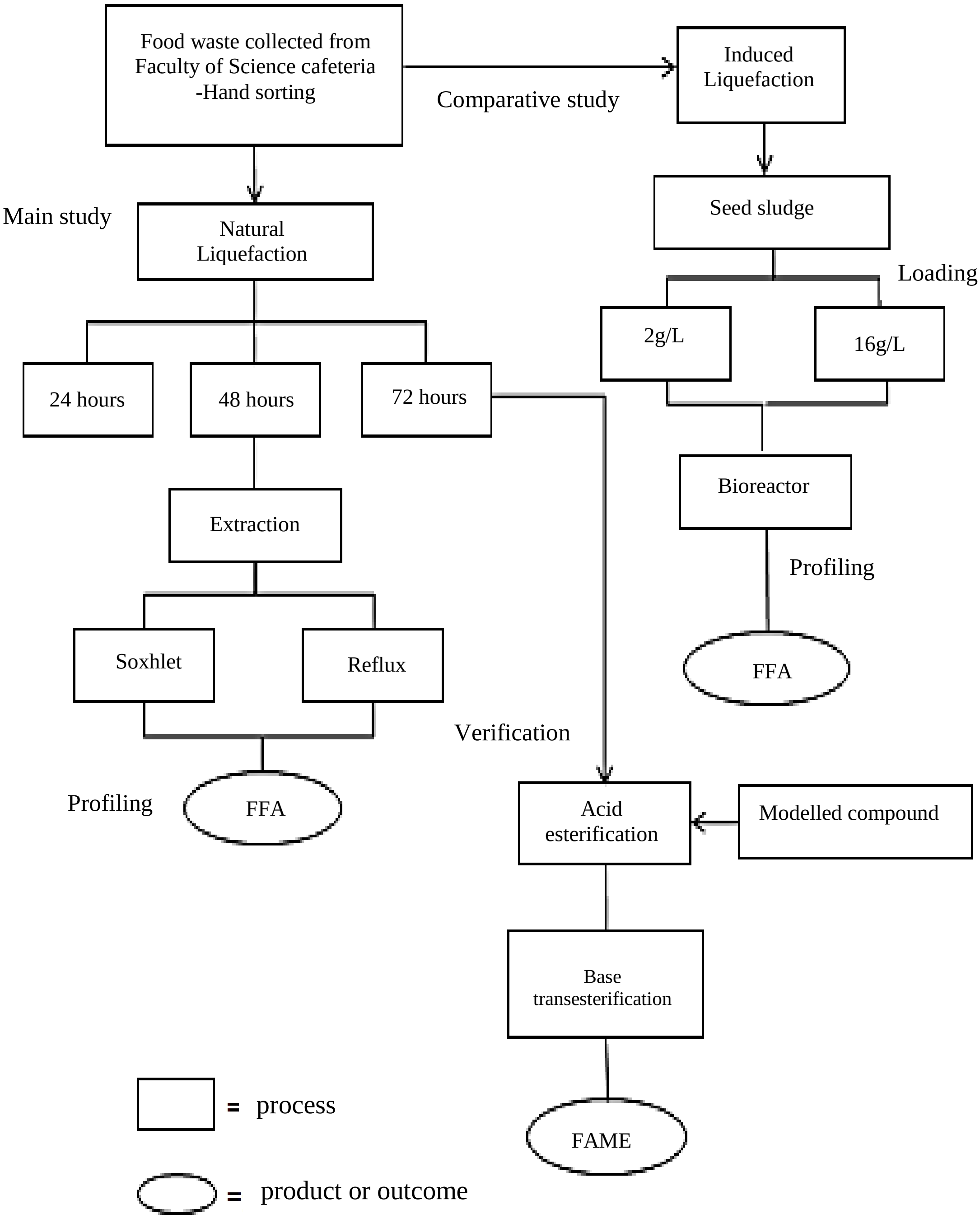


Figure 3.1: Schematic diagram of the process involved in the study

3.3 Preparation of Food Waste for FFA Extraction

The food waste was collected from Faculty Science cafeteria of University Malaya, Kuala Lumpur. The collected samples were sun-dried, and when it rained, the sample was kept in the fridge. After drying, all bones and shells were isolated from the food waste, before they are to be grind in order to provide smaller space for storage and bigger surface area.

3.4 Experimental Procedure

While the chemicals used in this study are listed in Table 3.1. All chemicals were analytical grade which were supplied by Merck Chemical Company, Germany.

Table 3.1: List of chemicals utilized for the extraction of FFA from food waste

Chemicals	**Grade**
n-Hexane	>99.9%
Dichloromethane	99.9%
Methanol	99.9%
Potassium	85.0%
Isopropanol	99.8%
Sulphuric Acid	98.0%

3.4.1 Extraction and Purification of FFA

3.4.1 (a) Solid-liquid extraction using Soxhlet

The food waste sample collected was hand sorted to separate inorganic solid such as plastics and Styrofoam before extraction. Extraction of food waste sample using solid-liquid extraction method was done with Soxhlet apparatus. Sorted food waste was dried in hot air oven for 24 hours at 180 ^{o}C before the extraction process. The moisture content of dry sample was calculated by Equation (3.1):

$$\frac{\text{Initial mass (g)} - \text{Final mass (g)}}{\text{Initial mass (g)}} \qquad \text{Equation (3.1)}$$

The dried sample was then crushed into smaller pieces by using mortar and pestles. Exact amount of 150 gram crushed sample was weighed and placed in the Soxhlet apparatus. Analytical grade of n-hexane and dichloromethane (with purity, 99%) weighed 300 gram were used as the solvent for extraction of FFA. Extraction using n-hexane and dichloromethane were done for 1 hour at 60 °C and 40 °C respectively.

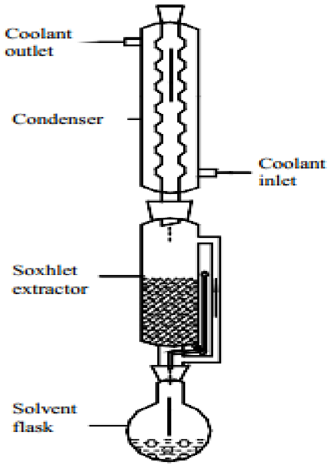


Figure 3.2: The set-up of Soxhlet apparatus

3.4.1 (b) Liquid-liquid extraction using Reflux

Reflux apparatus as shown in Figure 3.3 was used for liquid-liquid extraction by applying 1:2 ratio (weight over volume) of wet food waste sample. Again, two typical solvents of n-hexane and dichloromethane weighed 300 gram were used for extraction of FFA. Extractions were done for 1 hour at 60 °C and 40 °C respectively.

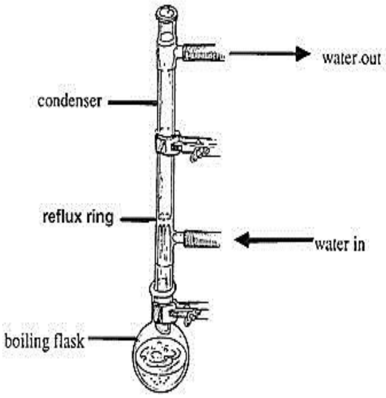


Figure 3.3: The set-up of Reflux apparatus

3.4.2 Food Waste Liquefaction

Natural liquefaction process by hydrolysing the substrate took place in the airtight-container for three duration time 24, 48 and 72 hours respectively. Water was added with the ratio 1:9 (substrate: water) and no additional microbial seed was used. Meanwhile for induced liquefaction, liquefaction of food waste was not done at the end of methane production. The methanization phase was not the focus of the work, therefore there was no indication of methane (CH_4) gas production during the liquefaction process. The study only involves the series of FFA production during acidification phase.

The introduction of seed sludge into the food waste was carried out at the end of methanogenesis when the secondary sludge taken from the sewage treatment plant (STP) had fully stabilized (digested). This can be observed when the formation of gaseous bubbles has stopped. It is important for the stabilized sludge to be used as the seed because it contains mostly the microorganism.

3.4.3 Modelled Substrates

Modelled substrates were designed for synthesis of methyl ester. These substrates of selected FFA consist of acetate, propionate, butyrate and valerate compound. The choices selection of these short chain fatty acids (C2-C5) as feedstock for biodiesel was based on the fact that SCFAs in free state or esterified to glycerol and can be fully converted to methyl esters. As SCFAs are volatile and soluble in water precautions at several stages of procedures were given attention to ensure its suitability as feedstock for biodiesel synthesis.

3.4.4 Synthesis of Fatty Acid Methyl Ester

3.4.4 (a) Acid Esterification

All experiments were performed using a 250ml three-neck round bottom flask with a condenser attached in order to avoid alcohol loses. The equipment assembly was placed on a heating plate with magnetic stirring. A thermometer was placed inside the neck of the round bottom flask to monitor and controlled the reaction temperature. The phases of reaction mixture were separated with separating funnel. The retained methanol and water in the product was then evaporated using a rotary vacuum evaporator. Feedstock oil from 72 hours self- hydrolysis food waste sample and modelled compound of SCFAs were poured into the three-neck round bottom flask and heated until the desired temperature according to the experimental methodology developed by Mishra et al. (2013) and Nakpong & Wootthikanokkhan (2010).

As the feedstock oil reached the desired temperature, mixture of alcohol (methanol) and catalyst (H_2SO_4) were poured into the flask. The reaction continues for specific reaction time. For all the experimental runs the magnetic stirrer speed was maintained at 400rpm. After the completion of the reaction the mixture was separated under gravity using separating funnel. The bottom layer was the desired product while the top layer contained excess methanol, glycerol and catalyst.

The desired product was washed with the deionized warm water until the washed solution reached pH 7. The remaining amount of water and methanol in the desired product was evapored under vacuum in the rotary evaporator. To ensure complete water removal from the product, 10 gram of sodium sulphate (Na_2SO_4) was added and the remained Na_2SO_4 was filtered from the product.

3.4.4 (b) Base Transesterifcation

Pretreatment oil from 72 hours self-hydrolysis food waste sample and modelled compound of SCFAs was poured into the three neck round bottom flask of 250ml. The flask was then placed in water bath with a condenser inserted in the center neck of the flask. The oil was warmed by adjusting the temperature of hot plate to 60°C. The required amount of methanol and potassium hydroxide (KOH) were poured into the flask. A mixing was done at 400 rpm with the required temperature was maintained at 180 min of reaction time. After completion of the reaction, mixture was poured into a separating funnel and it was allowed to separate under gravity.

The upper layer of fatty acid methyl esters was separated and washed with deionized warm water to remove impurities. 10 g of anhydrous Na_2SO_4 was added in the product to ensure removal of water before it was filtered.

3.5 Analytical Methods

3.5.1 FFA Profiling

In general, profiling the FFA is based on the retention time for each peak to be detected by the analytical instrument, in which this study used Gas Chromatography Mass-Spectrometry (GCMS) and Liquid Chromatography Mass-Spectrometry (LCMS). The profile also depends on the percentage of the peak area. The calculation of percentage (%) has been done as below:

GCMS – It used Agilent software based on below formula:

Example:

$$\frac{\text{Area}}{\text{Total peak area}} \times 100\%$$

Similarly, the calculation of area by LCMS is based on quantity of the peak areas for the single current over the total area occurred during analysis at the expected retention time.

3.5.1 (a) Gas Chromatography Mass-Spectrometry (GCMS)

Extracted FFA from the natural liquefaction of food waste was analysed using GCMS. The extracted oil soluble was filtered using glass syringe attached with disposable PTFE filter. Then the mixture of methanol consisted of 1.5ml oil extract and 1ml of 15% H_2SO_4 was placed in boiling tube. The tube was placed in heating block with temperature of 70 ^{o}C for 2 hours. After heating, 1ml of distilled water was added in the tube and the mixture was shaken vigorously. The solution was left for 1 hour, before the oil layer was separated. The oil layer was then diluted with n-hexane prior to injection into the gas chromatography. The fatty acids composition was quantified using Agilent 5975C inert MSD with tripleaxis detector and polyethylene glycol column. The temperature program was set to 40 ^{o}C for 3 minutes and was raised to 250 °C at a rate of

5 °C/min and was maintained for 49 minutes. Helium was used as carrier gas at constant flow of 1ml/min. Vegetable oil with concentration of 1000 ppb was used as standard.

3.5.1 (b) Liquid Chromatography Mass-Spectrometry (LCMS)

FAME and natural liquefaction sample of 24, 48 and 72 hours were determined by LCMS/MS method using AB Sciex 3200Q Trap LCMS/MS with Perkin Elmer FX 15 uHPLC system. Agilent Zrbax C18, 150mm x 4.6mm x 5µm was used as column. Two types of buffer were used; Type A : water with 0.1% formic acid and 5mM ammonium formate and Type B is Acetonitrile with 0.1% formic acid and 5mM ammonium formate. For the MS settings, the voltage IS and source temperature was set at 5500V with temperature of 50 ^{o}C each. The gradient run program was set at 10% B to 90% B from 0.01min to 0.8min. It was hold for 3 min and back to 10% B in 0.1min and re-equilibrated for 5 min.

The sample was diluted in 2mL solvent and filtered with nylon 0.22µM. Analysis was carried out by injecting 20µL of diluted sample solution.

3.5.1 (c) Gas Chromatography Flame Ionization Detector (GC-FID)

FFA obtained from induced liquefaction was analyzed using Agilent 7890A GC-FID equipped with capillary column coated with the polyethylene glycol as a stationary phase, the column of 30 m, internal diameter of 0.25 mm and thickness of 0.25 µm was used to determine the ester content. Approximately 100 mg of homogenized sample was weighed in a 10 ml sample tube and diluted with 10 ml of analytical grade toluene. A 1 µl of the solution was injected into the GC-FID and after prescribed time the chromatogram with different peaks were obtained. The identification of the modelled substrates standard peaks was compared with the retention time of sample peaks.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

This chapter contains the experimental results and discussion. It is represented in five sections. In the first section, it presents the results on preferred extraction method on wet sample and followed by the characterisation of FFA as the pure compound. The second section, it highlight the experimental results on feasibility of food waste to become raw substance for production of biodiesel synthesis. The third section, it presents FFA profiling from food waste as the raw substance in three different stages; fresh, natural liquefaction and induced liquefaction. The results also highlight the comparison of FFA composition of substrate derived from three different stages of natural liquefaction; 24, 48 and 72 hours respectively.

The last section on this chapter reports the findings on the viability of FFA as biodiesel feedstock by esterification process. The results discussed the verification of biodiesel production from two types of feedstock; 72 hours natural liquefaction food waste and modelled compound.

The experimental design is based on the preliminary study which was done on biodiesel which to verify the fact that fatty acids such as SCFAs in the free state or esterified to glycerol can be converted to methyl esters. All work for all samples were done in duplicate to reduce the source of error.

4.2 Selection of FFA Extraction Method

4.2.1 Waste moisture content

Using Equation in 3.1, moisture content of the food waste sample in this study was approximately 64%. The high moisture content is comparable to the moisture content obtained by Johari et al. (2012) in Malaysian food waste which it stated the range of 52-62%. This high moisture content can be a major problem if extraction is derived from fresh sample. In order to make FFA extraction in wet sample more effective, drying process would be necessary as pretreatment method. However the concern on pretreatment method is mainly on the extraction time.

4.2.2 Comparison of using Soxhlet and Reflux method

Food waste used in this study is a heterogeneous sample which present in a mixture form of animal fragments, vegetables, oils and water. For extraction and purification of FFA, the oil and grease need to be extracted from the food waste sample. The common method of oil extraction from solid sample is the solid-liquid extraction method using Soxhlet apparatus (Wang & Weller, 2006). However, this method has the disadvantage as the sample required to be dried for long hours before extraction. The drying step is required to remove the moisture in order to avoid influence of the moisture during FFA analysis.

In this study, Soxhlet method was adopted as standard and reference methods for suitability of Reflux method in extraction and purification of FFA. Reflux method was studied based on its potential in extraction and purification of FFA using wet sample, length of processing time, solubility of solvent in the method and also types of fatty acids extracted.

Organic solvents (dichloromethane and hexane) with low polarity level were chosen in both Soxhlet and Reflux for solubility of these solvents in extracting FFA. The weight of 150 gram food waste sample was used since it was the appropriate amount to fit in the apparatus. The food waste sample was used for the FFA profiling basis, not for the used of harvesting of FFA.

Comparative methods for Reflux and Soxhlet which are done in this study is to reflect what are the FFA profiles that could be acquired during extraction, not on the issue of how much FFA can be harvested.

All FFA profile analysis were carried out using GCMS analysis. Results of the GCMS analysis for the standard compound profile from vegetable oil are shown in Table 4.1. The standard which is used in this study was based on vegetable oil, therefore the consideration was on listed fatty acids where these fatty acids were the major fatty acids that generally occurred in food waste.

Table 4.1: GCMS analysis of standard compound profile from vegetable oil

Peak	Retention Time (min)	Fatty Acids
1	22.086	Myristic acid
2	26.942	Hexadecanoic acid
3	32.195	Octadecenoic acid
4	35.921	Octadecatrienoic acid

a) Results of using dichloromethane solvent

i) For Soxhlet method, analysis of GCMS shows the indication of C16 to C18 fatty acids in the range of 26.41 to 35.51 minutes. The fatty acids obtained from the study are shown in Table 4.2 with hexadecenoic acid (1.11%), hexadecanoic acid (69.54%), octadecenoic acid (16.43%) and octadecatrienoic acid (0.78%). No quantification of concentration was done since the aim was to see the profiling of the extraction sample. The area (%) refers to percentage of fatty acids composition. From the results, it can be observed Soxhlet method using dichloromethane solvent extracted higher amount of C16 fatty acids at retention time less than 30 minutes.

Table 4.2: GCMS analysis of fatty acids profile from Soxhlet extraction method using Dichloromethane

Peak	**Retention Time (min)**	**Percentage (%)**	**Fatty Acids**
1	26.41	1.11	Hexadecenoic acid
2	27.00	69.54	Hexadecanoic acid
3	30.73	16.43	Octadecenoic acid
4	35.51	0.78	Octadecatrienoic acid

ii) For Reflux method, analysis of GCMS also shows the indication of C16 to C18 fatty acids in the range of 26.94-31.94 minutes. The fatty acids obtained from the study are shown in Table 4.3 with hexadecanoic acid (0.18%), octadecenoic acid (18.65%) and octadecanoic acid (31.94%). It can be observed Reflux method using dichloromethane solvent extracted lower amount of C16 fatty acids at retention time less than 30 minutes.

Table 4.3: GCMS analysis of fatty acids profile from Reflux extraction method using Dichloromethane

Peak	**Retention Time (min)**	**Percentage (%)**	**Fatty Acids**
1	26.94	0.81	Hexadecanoic acid
2	31.13	18.65	Octadecenoic acid
3	31.94	2.37	Octadecanoic acid

b) Results of using n-hexane solvent

i) For Soxhlet method, analysis of GCMS shows the indication of C16 to C20 fatty acids in the range of 27.83-35.97 minutes. The fatty acids obtained from the study are shown in Table 4.4 with arachidonic acid (96.0%), hexadecenoic acid (42.25%) and octadecanoic acid (6.96%). From the results, it can be observed Reflux method using n-hexane solvent extracted high amount of C20 fatty acids at retention time 35.78 minutes.

Table 4.4: GCMS analysis of fatty acids profile from Soxhlet extraction method using n-hexane

Peak	Retention Time (min)	Percentage (%)	Fatty Acids
1	35.78	96.00	Arachidonic acid
2	35.97	42.25	Hexadecenoic acid
3	32.61	6.96	Octadecenoic acid

Qualitative analysis of GC-MS relies on comparison of the peaks retention time in an unknown sample and known standard. There are a few factors which may interrupt the retention time of the unknown sample when compared to the known standard, i.e. delay on the analytical work. Huge gap for the concentration between known and unknown sample also can influence the RT although, the same GC and column were to be used. RT of sample increased due to the different concentration used in both standard and sample. Standard concentration used in this study was higher (1000 ppb) compared to sample (500 ppb). The different in both concentrations have caused interaction problem of active site on polarity stationary phase contain functional groups such as hydroxyl group (-OH) during analysis (Skoog et al., 1999)

ii) For Reflux method, analysis of GCMS shows the indication of C16 to C20 fatty acids in the range of 27.55-38.01 minutes. The fatty acids obtained from the study are shown in Table 4.5 with hexadecanoic acid (64.36%), octadecanoic acid (9.72%), arachidonic acid (6.95%) and eicosanoic acid (3.45%). It can be observed Reflux method using n-hexane solvent extracted lower amount of C20 fatty acids at retention time of 36.01 minutes.

Table 4.5: GCMS analysis of fatty acids profile from Reflux extraction method using n-hexane

Peak	Retention Time (min)	Percentage (%)	Fatty Acids
1	27.55	64.36	Hexadecanoic acid
2	31.52	9.72	Octadecanoic acid
3	36.01	6.95	Arachidonic acid
4	38.01	3.45	Eicosanoic acid

Table 4.6 shows two identical types of fatty acids; hexadecanoic acid and octadecenoic acid using Soxhlet and Reflux method. Soxhlet has been a standard technique used for extraction and it has been a reference when a new extraction method such as Reflux is developed. Extraction using Reflux method was evaluated based on its potential of using wet sample, length of processing time, solubility of two solvents; dichloromethane and n-hexane in the extraction and the types and amount of fatty acids extracted.

It was observed similar times of fatty acid identification peaks were recorded in both methods. In reference to the FFA percentage, higher oil yield was observed in extraction process with Soxhlet extraction. The result showed 29% and 10% different of weight percentage in both methods respectively. Different of weight percentage can be attributed to the drying of the sample prior to extraction process using Soxhlet method. Pretreatment with long drying process reduced moisture and volatile content in sample, thus increased the extraction of polar substance i.e. FFA. It has been reported that drying time of the seeds is an important factor in Soxhlet method in producing higher oil yield (Bokhari et al., 2012).

Table 4.6: Comparison of retention time identical fatty acids in Soxhlet and Reflux method by GCMS analysis

	Retention Time (min)	
Fatty Acids	**Soxhlet**	**Reflux**
Hexadecanoic acid	27.83	27.55
Octadecenoic acid	32.61	31.52

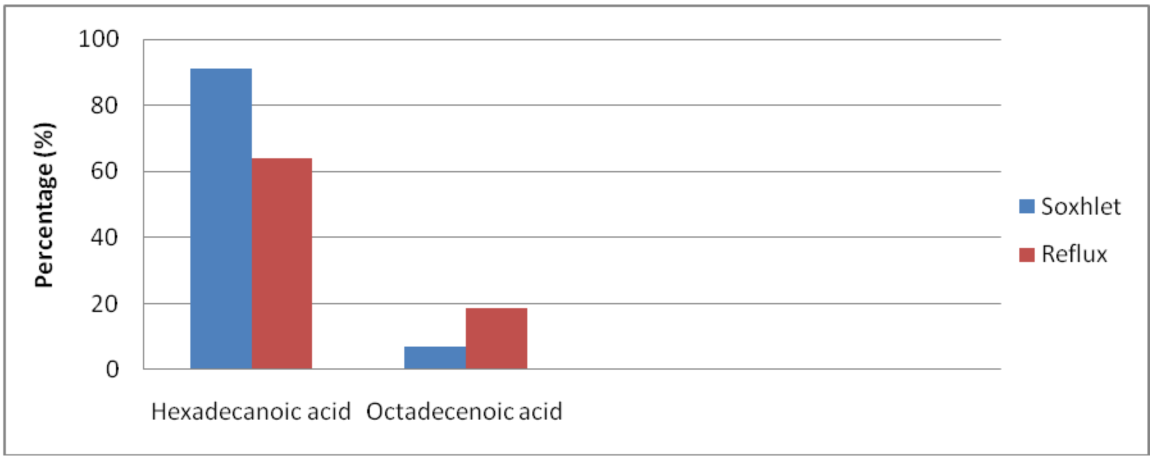


Figure 4.1: Comparison of percentage on two identical fatty acids detected by Soxhlet and Reflux method

Extraction process significantly influences by polarity and sample to solvent ratio (Amin et al., 2012; Bokhari et al., 2012). This fact can be supported with the results obtained. The results from two different solvent in two extraction methods shows different effectiveness on fatty acids extraction. Both Soxhlet and Reflux method with dichloromethane extracted similar type of fatty acids. Extraction using this polar solvent extracted polar lipids (hexadecenoic, hexadecanoic, octadecenoic and octadecatrienoic). As dichloromethane is polar solvent, it is likely to extract polar compound. While extraction using non-polar solvent, n-hexane indicates the mixture composition of polar lipid, triglycerides and neutral lipids. The results suggested that n-hexane is more effective at extracting lipid from dried sample which in good agreement with report by Jones et al. (2012). The authors stated effectiveness of hexane in extracting lipid is based on the type of sample. Hexane is more effective at extracting lipids from dried sample compared to wet sample.

The used of 1:2 sample to n-hexane solvent ratio in Reflux method results in lower weight percentage of fatty acids typically for saturated fatty acids. Figure 4.2 shows independent results of extraction using Soxhlet and Reflux method. The result indicates that presence of water in Reflux sample decrease the solvent polarity interaction. Direct heating during refluxing also suggests that some loses of FFA may have taken place during the heating place.

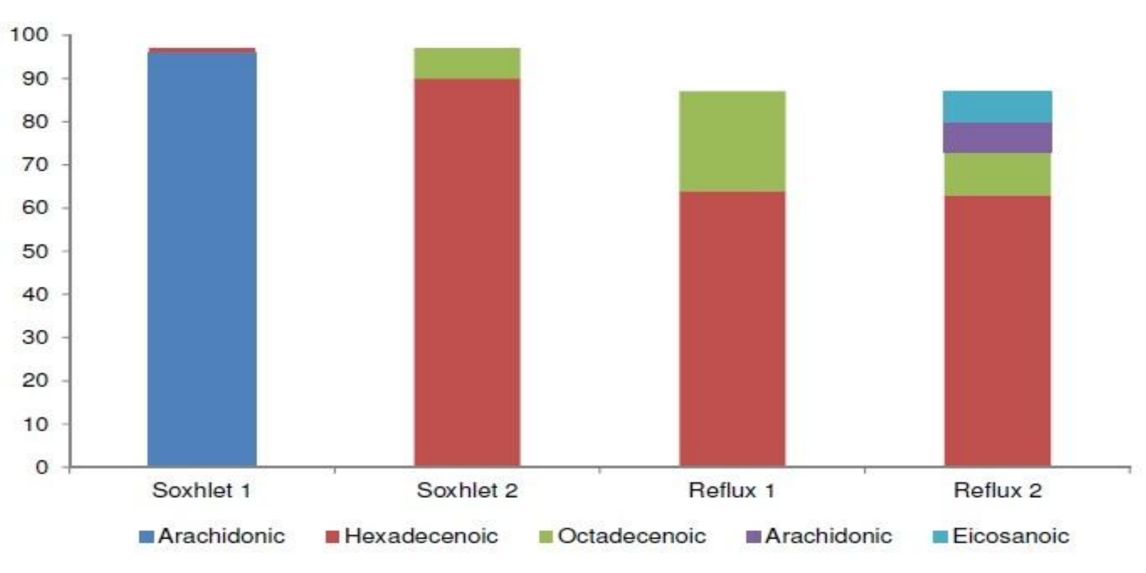


Figure 4.2: Comparison of fatty acids composition by Soxhlet and Reflux method

Although extraction using a solid-liquid by Soxhlet has been established for lipid extraction, there are few disadvantages with the method. Long process of extraction, high volume of solvent, possibility of thermal decomposition and the lack of agitation to accelerate the process generally made the Soxhlet method inappropriate for shorter processing time and cost effective extraction method (Wang & Weller, 2006).

Drying is the most popular pretreatment applied when dealing with food waste extraction before application of solid-liquid extraction method using Soxhlet (Angel et al., 2012; Rakib et al., 2013; Nakpong & Wootthikanonokkhan, 2009). Since the aim of this study is to develop the extraction method to resolve the issue of long hour extraction method using the conventional Soxhlet, pretreatment steps for the sample was no longer required and liquid-liquid extraction method using Reflux was applied. Reflux method was applied based on the successful of oil extraction from soybean seeds using trichoethylene solvent (Nikolić et al., 2009).

As food waste sample in this study present in high amount of water content, hence it would ideal to extract the oil without having to remove the water from the sample. Reflux method based on liquid-liquid extraction theory was developed since application of this method on wet sample in extracting FFA is possible and could develop better extraction than Soxhlet method. Similar fatty acid identification of C16 to C20 was recorded in both methods with higher percentage oil was observed in Soxhlet method. The different values of 29% and 10% on hexadecanoic and octadecanoic acids respectively indicates that on economic point of view, Reflux extraction method could be enhanced for a sustainable industrial process.

Elimination step of drying process in Reflux method will shorter the processing time of extraction, thus overcome the shortcoming of Soxhlet method. The elimination step of drying also affects the whole process economy as by maintaining the moisture content in food waste sample it will save energy required to dry.

4.3 Food Waste as the Source for FFA

4.3.1 FFA profiling of fresh hydrolysed food waste

FFA extraction was done at early stage of liquefaction where it occurs during hydrolysis and acidification of complex substance in the food waste. The substance in the food waste is excluding the bones, and shell or other substance which unable to be hydrolysed. Extraction of FFA was based on principle of isolating an analyte from solid sample (food waste) in hexane solvent without drying process at the earlier stage.

Food waste used in this study consists of animal and plant residues where it contributed to different type of fatty acids composition. Therefore it can be expected that fatty acids composition in food waste sample used would vary in different stages of liquefaction period since the length of hydrolysis process will mainly affected the biological process and product obtained.

Figure 4.3 shows the LCMS analysis of fatty acids on liquefaction periods of 24 hours fresh sample. Two types of fatty acids were detected, which are linolenic acid and myristoleic acid.

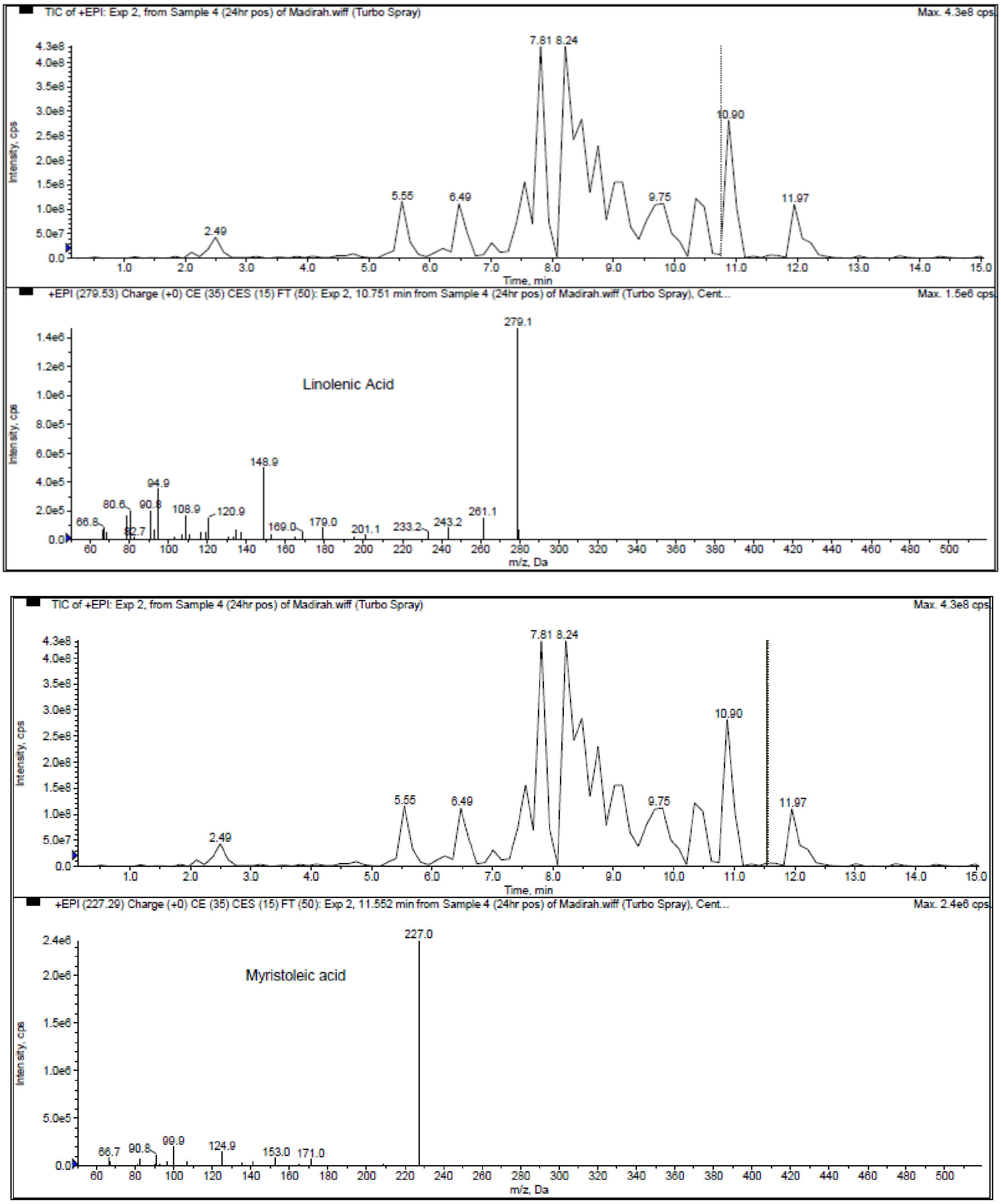


Figure 4.3: LCMS analysis of linolenic and myristoleic acid of fresh 24 hours liquefaction food waste

Figure 4.4 and 4.5 shows the LCMS analysis of fatty acids on liquefaction periods of 48 hours and 72 hours fresh hydrolysed food waste. Both samples indicate the present of myristoleic acid. All three liquefaction periods indicate polyunsatured fatty acids (myristoleic acid). It was noticed in 24 hours sample, additional type of fatty acids which is linoleic acid was detected in the sample. These results show that self-hyrolysis of food waste solubilised the waste matter and accumulated the polyunsaturated fatty acids (linoleic and myristoleic). The results obtained are comparable to the results in application of commercial enzymes for hydrolysis of food waste and organic waste (Pleissner & Lin, 2013)

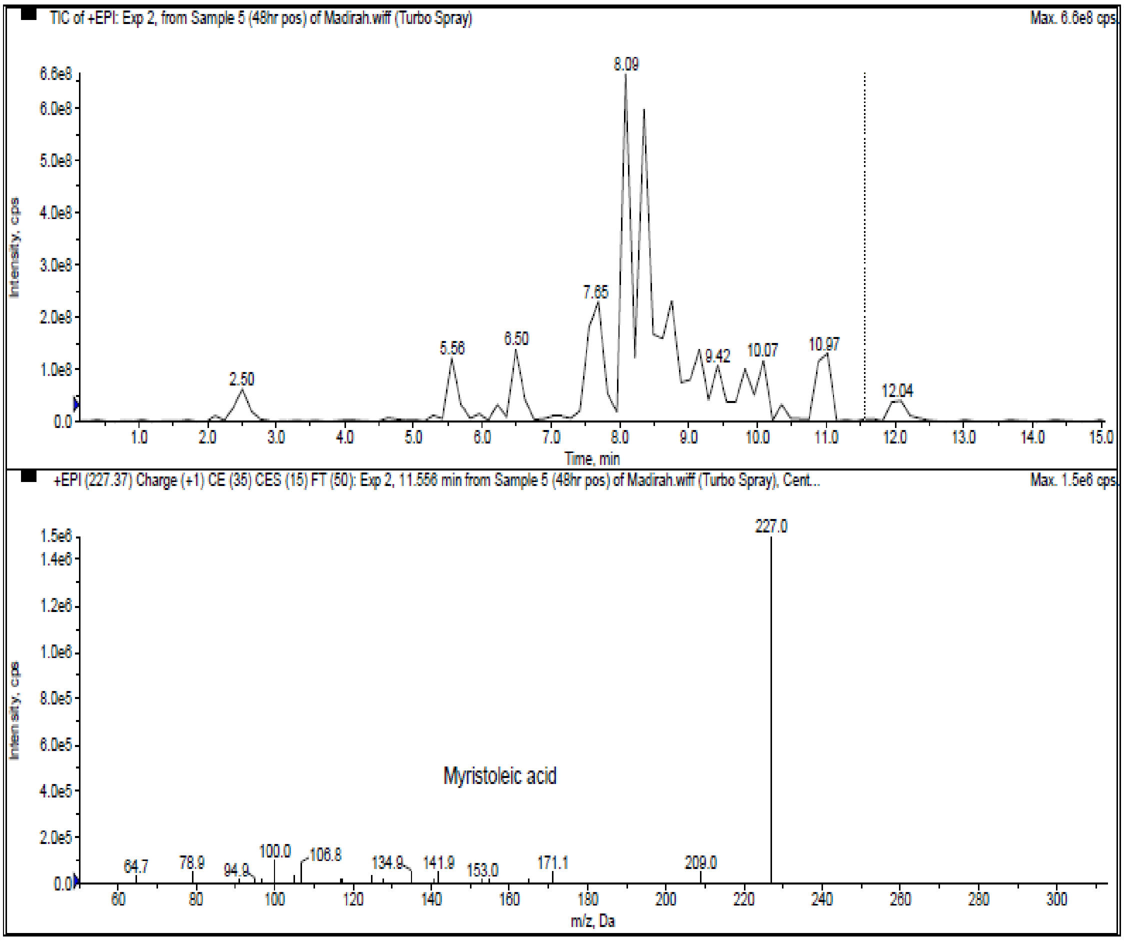


Figure 4.4: LCMS analysis of myristoleic acid of fresh 48 hours liquefaction food waste

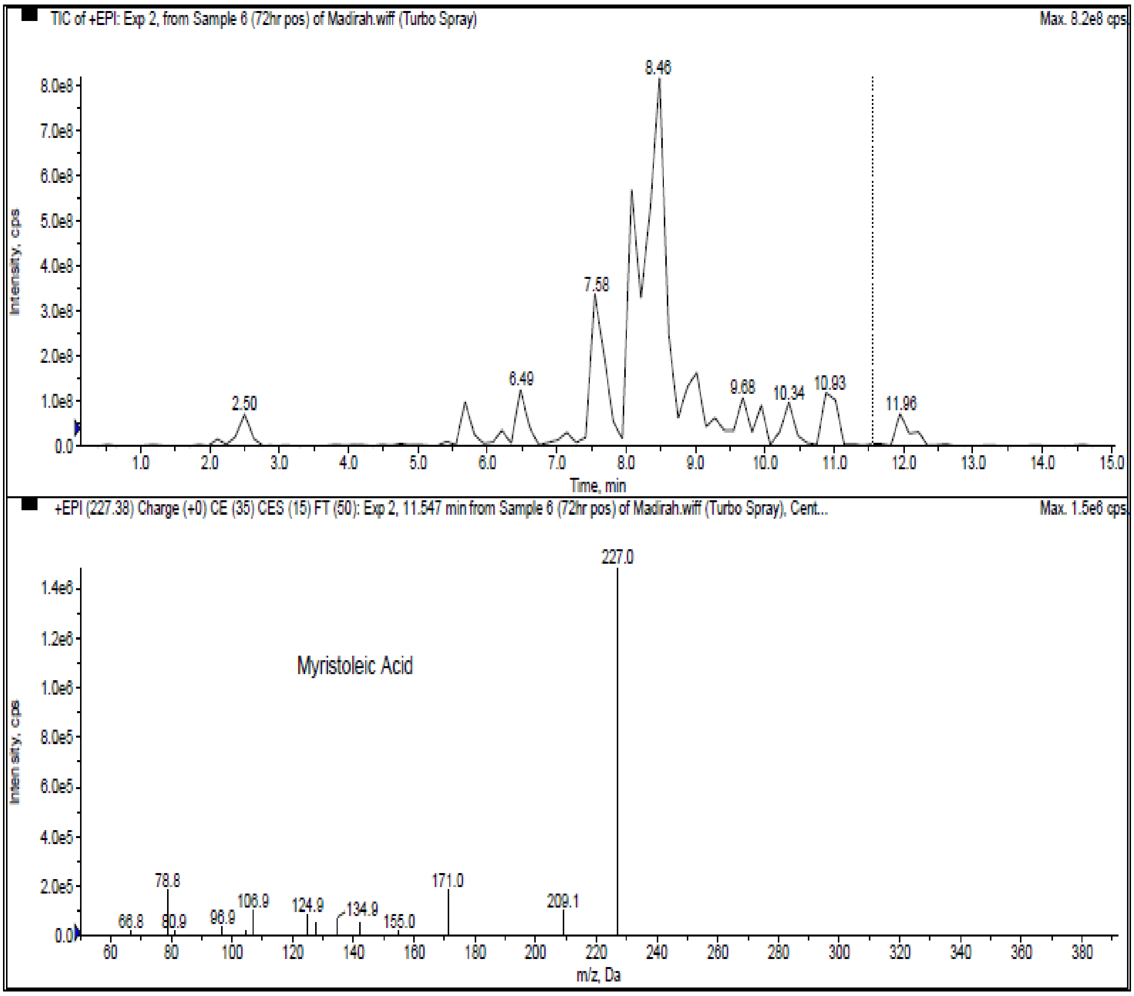


Figure 4.5: LCMS analysis of myristoleic acid of fresh 72 hours liquefaction food waste

4.3.2 FFA profiling on liquefaction periods of food waste

Table 4.7, 4.8 and 4.9 show the fatty acids profile of oil extraction on different period of liquefaction food waste (24, 48 and 72 hours). Results indicate both samples content fatty acids carbon ranging from C15-C18. The obtained results show that the fatty acids composition consists of saturated fatty acids such as pentadecanoic, hexadecanoic, octadecadienoic and octadecenoic acid. These findings are in agreement with the predominant hydrolysis products observed by Silva et al, (2013). It was reported predominant hydrolysis products are acetic, propionic and n-butyric acids for all the organic waste streams (olive mill effluent and winery effluent).

Silva et al, (2013) also mentioned a small amount of n-valeric (pentanoic acid), iso-valeric and n-caproic acids were observed in sample obtained from landfill. However, it was observed in this study fatty acids with carbon less than five were not observed in both hydrolysed food waste period. The undetected carbon-3 (C3) fatty acids in this study possibility due to hydrolysis were carried out naturally compared to the study done by Silva which the hydrolysis was done in bioreactor.

In theory, during hydrolysis of organic matter such as cellulose, hemicelluloses, lignin and pectin become part of the liquid phase given the action of acidogenesis that provide extracellular enzymes reaction. In this study, the shifting phase to anaerobic environment occurred when the sample was hydrolysed in an enclosed container. Acidification phase was likely to take place during liquefaction process. In this condition, oxygen is depleted by microorganism respiration. This degradation process affected the chemical oxygen demand (COD), VFA and CO_2 production. The continuous hydrolysis of food waste followed by the microbial conversion of biodegradable organic content resulted in high production of FFA typically VFA and COD.

The result in Figure 4.6 shows analysis of fatty acid profile for three different stages of natural liquefaction of food waste. The high percentage of VFA is recorded in 24 hours liquefaction period. The result recorded clearly indicates the favourable condition for the growth and activity of acid-producing microorganisms. High amount of pentadecanoic acid also indicates extended hydrolysis of the particulate material into soluble substances and acidogenesis of solutes into VFAs.

The reducing trend of retention time (min) in all fatty acids was observed in 72 hours liquefaction period. This opposite trend could be related to the changing of acid phase in the sample. It can be observed the time interval from 24 hours to 72 hours recorded the decreasing amount of fatty acids carbon-5 (pentadecanoic acid).

Decreasing of the VFA is possibility due to VFA is consumed and converted to methane gas.

Table 4.7: GCMS analysis of fatty acids profile from 24 hours hydrolysed food waste

Sample	Peak	Retention Time (min)	Percentage (%)	Fatty Acids
No. 1	1	1.95	1.98	Pentane
	2	2.10	61.99	Pentane
	3	2.27	5.69	Cyclopentane
No.2	1	2.25	14.99	Pentane
	2	2.37	15.75	Pentadecanoic acid
	3	28.47	1.79	Hexadecanoic acid
	4	32.99	1.39	Octadecanoic acid

Table 4.8: GCMS analysis of fatty acids profile from 48 hours hydrolysed food waste

Sample	Peak	Retention Time (min)	Percentage (%)	Fatty Acids
No. 1	1	1.98	1.85	Pentane
	2	2.20	42.11	Pentane
	3	2.25	5.60	Cyclopentane
No.2	1	2.27	14.89	Pentane
	2	2.40	14.70	Pentadecanoic acid
	3	28.50	1.78	Hexadecanoic acid
	4	33.00	1.37	Octadecanoic acid

Table 4.9: GCMS analysis of fatty acids profile from 72 hours hydrolysed food waste

Sample	Peak	Retention Time (min)	Percentage (%)	Fatty Acids
No. 1	1	2.23	10.57	Pentane
	2	2.38	13.45	Pentadecanoic acid
	3	28.51	7.47	Hexadecanoic acid
	4	32.81	1.78	Octadecadienoic acid
	5	33.05	7.96	Octadecenoic acid
No.2	1	2.25	14.99	Pentane
	2	2.42	15.75	Pentadecanoic acid
	3	28.51	1.79	Hexadecanoic acid
	4	32.73	1.39	Octadecadienoic acid

Table 5.0: GCMS analysis of fatty acids profile on three liquefaction period of food waste

Fatty Acids	**24 hours liquefaction food waste Retention time (min)**	**48 hours liquefaction food waste Retention time (min)**	**72 hours liquefaction food waste Retention time (min)**
Pentadecanoic acid	2.37	2.40	2.38
Hexadecanoic acid	28.47	28.50	28.51
Octadecadienoic acid	-	-	32.81
Octadecenoic acid	32.99	33.00	33.05

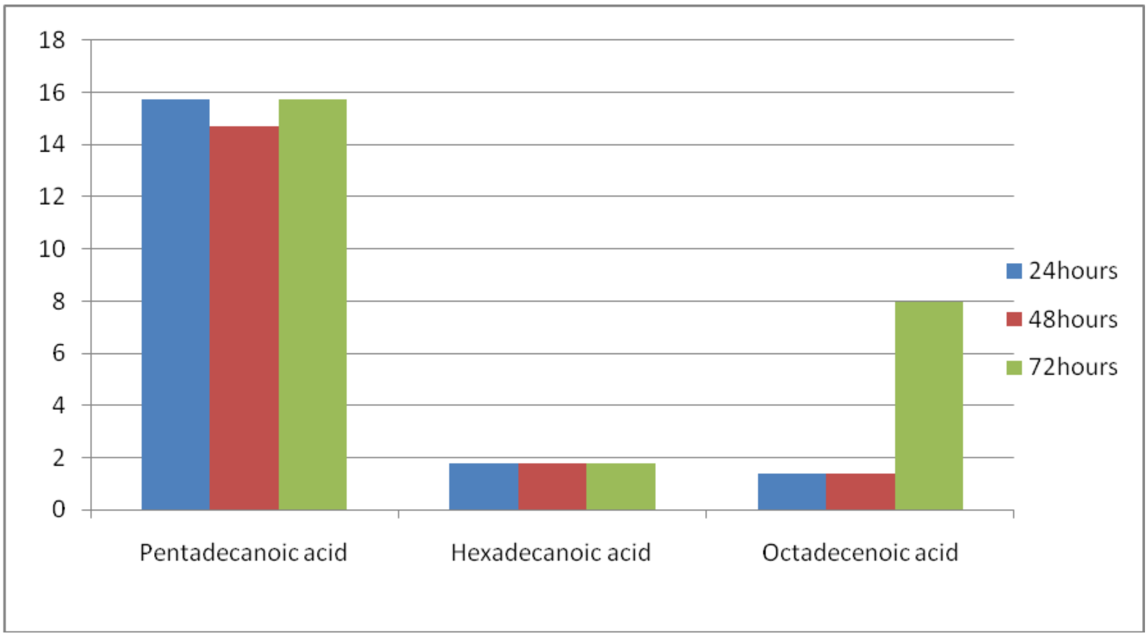


Figure 4.6: Percentage of fatty acids composition of three liquefaction period of food waste sample

4.3.3 Induced Liquefaction on fresh food waste sample with seed sludge

4.3.3.1 FFA profiling on mass loading

Extracted FFA from the hydrolysed liquefaction of food waste was analysed using GCMS. Salts of acetate, propionate, butyrate and valerate were used to represent VFA (C2-C6) as modelled compound. While for long FFA, this study utilized palmitic acid (C16), linoleic acid (C18), monolenolenin (C18) and monoolein (C21). Analysis using GCMS has been applied to quantify the fatty acids since it is suitable to quantify fatty acid with isomer C8 until C26 (Salimon et al., 2014; Bigelow et al., 2011).

Table 5.0 shows the retention time and area percentage recorded for the standard used for induced liquefaction on fresh sample with seed sludge. It recorded the standard which consists of carbon-15 and carbon-16 fatty acids in 3 to 4 minutes of retention time. Mass loading of the fresh food waste sample was made in two loadings; 2 gram/liter and 16 gram/liter. Each loading of fresh food waste was done in 24 hours time interval together with addition of seed sludge in bioreactor. For both loadings, composition of LCFAs had been found to be ranging from C15 to C16. The results on fatty acids elution was comparable with the results obtained by Katsuwon et al., (2006). Comparison of the retention time of the standard with the prediction time (t_R) equation reported in the report stated fatty acids C15 and C16 retention time is in the range of 3 to 5 minutes.

Table 5.1 presents the GC-FID results of mass loading of 2 gram/liter fresh food waste into the liquefaction chamber with seed sludge. The use of GC-FID instrumentation was to replace the earlier analysis using GC-MS when it was found that food waste sample main composition was watery form. GC-FID was used due to its suitability of sample analysis. It can be observed, the fatty acids with carbon-15 and carbon-16 was detected in the range of 3 minutes. In corresponding to area percentage, carbon-15 fatty acids recorded a high percentage compared to carbon-16 fatty acids. It is also noticed in Table 5.2 when the mass loading was increased to 16 gram/liter, the detected fatty acids (carbon-15 and carbon-16) was still detected in the range of 3 minutes with carbon-16 recorded the higher area percentage.

Table 5.1: GC-FID analysis of fatty acids profile from standard compound

Peak	Fatty Acids	Retention Time (min)	Percentage (%)
1	C15	3.178	0.002
2	C15	3.560	0.005
3	C16	3.736	0.015
4	C16	4.236	0.226

Table 5.2: GC-FID analysis of fatty acids profile from 2 gram/liter mass loading

Peak	Fatty Acids	Retention Time (min)	Percentage (%)
1	C15	3.028	99.90
2	C16	3.637	0.0012
3	C16	3.789	0.098

Table 5.3: GC-FID analysis of fatty acids profile from 16 gram/liter mass loading

Peak	Fatty Acids	Retention Time (min)	Percentage (%)
1	C15	3.026	99.89
2	C16	3.635	0.0012
3	C16	3.786	0.102

In the results, high percentage with 99.9% and 99.89% respectively were recorded for C15 composition in both food waste mass loading sample. Loading was done only for two consequent days due to the possibility that the fatty acids compound will remain to be the same although the food waste mass loading was increased. The indication of LCFAs in this study indicates that the degradation of LCFAs concentration occurred.

This finding is disagreeing with the report produced by Oh & Martin (2010). The report stated production of LCFAs caused major problem in treatment of oily or fatty wastewater. Production of LCFAs contributed to the generation of floaty microbial and inhibition of anaerobic microorganisms. As a result it could cause operational problem in the wastewater treatment process.

The findings in this study could be explained by an increase in mass loading could cause fat, oil and grease accumulated in seed sludge and increased the foam production as suggested by Cavaleiro et al. (2008).

4.4 Esterification of FFA and Modelled Compound

4.4.1 Esterification of modelled compound

Figure 4.7 shows analysis of fatty acid methyl ester under base esterification of modelled compound. As mentioned in Chapter 3.4.3, the modelled compound was designed with a mixture of pure compound of short chain fatty acids. Esterification process of this compound salts (acetate, propionate, butyrate and valerate) produced mixtures of fatty acid methyl ester in the range of C16 to C18.

FAME products were detected by mass spectrometer (MS) in LCMS system. This detection occurred when ionizes of each compound elutes and measures by the mass-to-charge ratio. In LCMS, chromatogram produced by the MS is known as total ion chromatogram (TIC). Chromatogram peaks of the FAME compound were identified by comparing the retention times to study done by Murphy (2012). Relative percentage of the product obtained was calculated based on quantity of the peak areas for single current signals over the total peaks area occurred during analysis at the expected retention time. The product obtained consists of methyl lenolenate, methyl linoleate, methyl oleate and methyl palmitate with relative percentage calculated as 71%, 35%, 20% and 71% respectively.

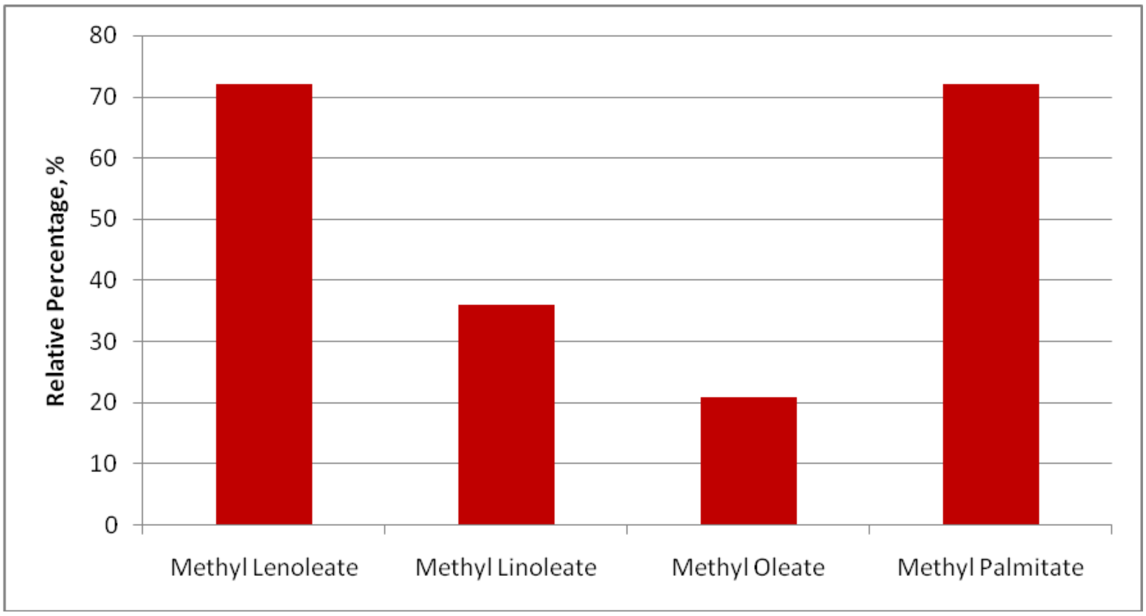


Figure 4.7: Analysis of fatty acid methyl ester under base esterification of modelled compound

Overall the relative percentage obtained from esterification of modelled compound produced high saturated fatty acids methyl ester compared to unsaturated fatty acid methyl ester. The used of methanol in esterification generally give higher conversions of esterification process (Kraai et al., 2008). However it can be noticed that high saturated fatty acids content was observed when using modelled compound. The compound of C2-C5 in the modelled compound contributed to high FFA. Low relative percentage was obtained due to high FFA that ineffectively transformed SCFAs to methyl ester.

High FFA also results in low kinetics rate of esterification process. This occurred as longer time needed for the FFA to be converted to methyl ester. Longer reaction rate produced lower FAME. Jain et al. (2011) stated rate of esterification reaction varies linearly with FAME yield.

Acyl chain length could also contribute to kinetics rate in esterification process. Increase of acyl chain in modelled compound from C2-C6 decrease the kinetics rate of esterification process as reported by Brault et al. (2014).

4.4.2 Esterification of FFA from food waste

Figure 4.8 shows analysis of fatty acid methyl ester for the sample taken from 72 hours of hydrolysis process. The esterification process of FFA was carried out under base catalyst using KOH. Analysis of the product was done using LCMS. Result indicates a mixture of saturated and unsaturated fatty acid methyl ester in the range of C16-C18. The relative percentage product obtained consists of methyl lenoleate (80%), methyl linoleate (91%), methyl oleate (93%) and methyl plamitate (40%). These relative percentages of FAME were calculated based on quantity of the peak areas for single current signals over the total peaks area occurred during analysis at the expected retention time.

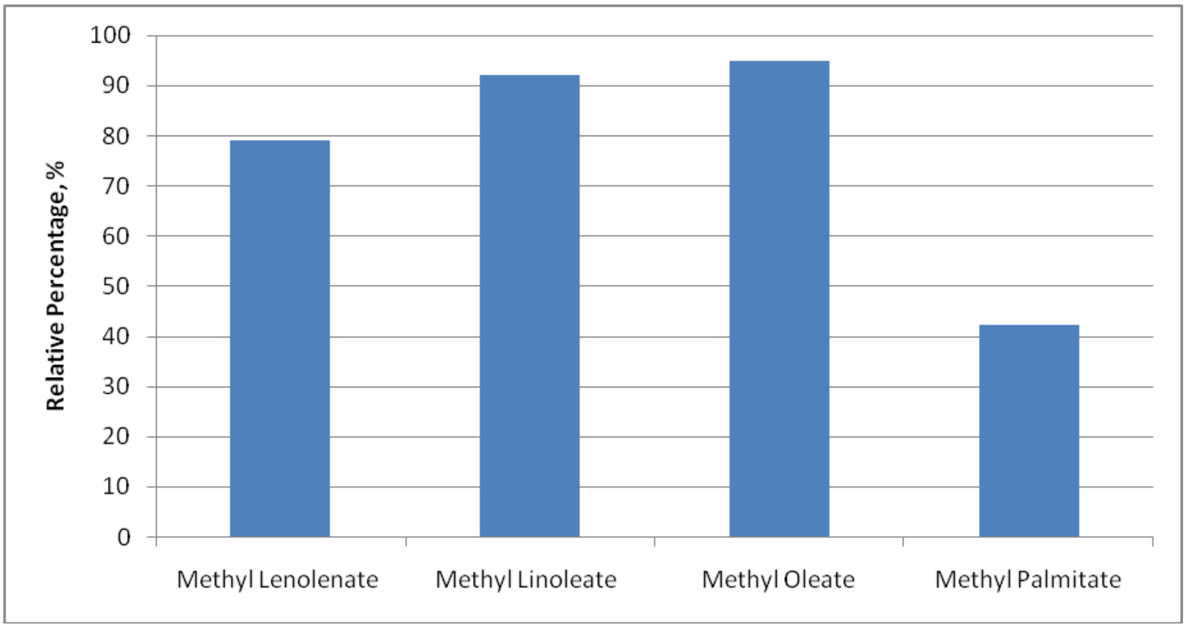


Figure 4.8: Analysis of fatty acid methyl ester under base esterification of FFA from food waste

The results of relative percentage of FFA esterification from food waste indicate that during base catalyst transesterification, methyl ester was obtained in 180 min when reaction temperature was 60 °C. It also noticed the results from this study comparable with the results reported by Hung et al. (2010). The authors reported that waste vegetable oil biodiesel produced methyl palmitate, methyl oleate and methyl linoleate with relative percentage in the range of 27-37%. Generally by comparison, the relative percentage of the FAME obtained from this study is high compared to the FAME product from waste cooking oil and virgin vegetable oil reported by Shalaby et al. (2012) and Hung et al. (2010). Both studies indicate PUFAs can be easily decomposed. This could be related to the oxidation stability of the substrate. From the results obtained in this study, it can be clearly observed that fatty acids with polyunsaturated double bonds have poor oxidation stability therefore produced lower amount of product.

Esterification of food waste in this study produced the lower amount of methyl palmitate. Higher amount of product yield can be obtained by maximized the shifting of the reaction product (ester and glycerin) as recommended by Hossain et al. (2010). Therefore in order to achieve higher amount of methyl palmitate in this study, the most appropriate technique is to increase the alcohol to oil ratio concentration as suggested by the literature. Initially the alcohol to oil ratio applied in this study was 3:1 with 1% by weight catalyst which is lower if compared to other study carried out with different sample type such as microalgae and sunflower oil sample (Angel et al., 2010; Hossain et al., 2010).

In term of catalyst, base catalyst using KOH was applied in this study. Since the saponification (formation of soap) in food waste sample is high due to its FFA content, application of KOH was to avoid the saponification process. In general, saponification will take place when FFA is to react with homogeneous base catalyst and form soap as describe in Equation (4.1). The next step will be done in alcoholic base catalyst and formed FAME. The process is describes in Equation (4.2).

$$\underset{\text{[FFA]}}{R_1 - COOH} \quad + \quad \underset{\text{Potassium Hydroxide}}{KOH} \quad \longrightarrow \quad \underset{\text{Soap}}{R_1COONa} \quad + \quad \underset{\text{Water}}{H_2O} \text{ ....Equation (4.1)}$$

$$\underset{\text{[FFA]}}{R_1 - COOH} \quad + \quad \underset{\text{Alcohol}}{ROH} \quad \longrightarrow \quad \underset{\text{FAME}}{R\text{-}O\text{-}CO\text{-}R_1} \quad + \quad \underset{\text{Water}}{H_2O} \text{ ....Equation (4.2)}$$

The used of base catalyst instead of acid catalyst in process is based on certain factors discussed in most literature. KOH was chosen due to its reaction process where it was reported base catalyst give a faster reaction compared to acid catalyst (Ganaprakasam et al., 2013; Mishra et al., 2013; Nakpong & Wootthikanokkhan, 2010).

As this study was done in laboratory scale, low temperature is required to suit the process. Therefore, base-catalysed was used because it was reported suitable to be used in low temperature (60 °C) and the conversion to methyl ester is direct with no intermediate steps (Ejikeme et al., 2010).

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

Biodiesel conversion and its feedstock from edible oil has lead to the concern that the need for fuel is being placed ahead over the need for food. The need to improve eco-sustainability of the energy production process encourages research on biodiesel production from wastes and renewal biomass. The main concern of biodiesel production from waste product is on the efficiency of oil extraction process due to its high percentage of lipid content.

Reflux method shows that application of this method on wet sample in extracting FFA is possible and could develop better extraction than Soxhlet method. Similar fatty acid identification of C16 to C20 was recorded in both methods with higher percentage oil was observed in Soxhlet method. The different values of 29% and 10% on hexadecanoic and octadecanoic acids respectively indicates that on economic point of view, Reflux extraction method could be enhanced for a sustainable industrial process.

Elimination of drying step in Reflux method could shorten the extraction process and reduce the energy consumption cost in the process. Extraction of FFA from food waste using n-hexane shows 84.48% compared to dichloromethane 21.83%. This shows that organic solvent of n-hexane is more efficient in extracting FFA compare to dichloromethane solvent.

The extraction of FFA in hexane solvent from three stages of natural liquefaction (24, 48 and 72 hours) indicates the present of myristoleic acid. This indication shows that self-hydrolysis of food waste solubilised the waste matter and accumulated the polyunsaturated fatty acids.

As addition to natural liquefaction findings, sludge was added as catalyst to enhance the hydrolysis of food waste. The profiling shows production of LCFAs with carbon-15 was the highest. This shows that increasing the mass loading (from 2g/liter to 16g/liter) of induced liquefaction food waste sample will not affecting the FFA amount and its composition.

As far as the project is concerned no production of biodiesel is intended in this study. However the study showed that natural liquefaction produced higher amount of biodiesel precursors, instead of using seed sludge as inoculums. Natural liquefaction produced better precursors, in term of volume and quantity compared to induced liquefaction.

Verification of the feedstock as biodiesel on 72 hours natural liquefaction sample and modelled compound produced mixtures of fatty acid methyl ester in the range of C16 to C18. High saturated fatty acid methyl ester was observed in esterification of FFA from modelled compound. However, the opposite trend was recorded in esterification process using FFA from hydrolysis food waste where the result indicates high unsaturated fatty acid methyl ester in the process. Viability of the feedstock was based on its economical and previous study done on same feedstock. Quality of the biodiesel produced was not analysed as the main objective was to determine its potential to be the alternative feedstock for biodiesel.

In conclusion, fresh and natural liquefaction food waste produced high recovery of FFA compared to induced liquefaction. Due to this, it is possible to use this fresh and natural liquefaction as the feedstock for synthesis of FAME.

For future work, it is recommended the hydrolysis of fresh food waste should be done in shorter time such as 6 and 12 hours. Environment factors such as temperature and pH need to be closely monitored to determine the relationship of those factors and production of FFA.

In corresponding to the higher of FAME yield through esterification process, few chemical factors related to the process need to be given special attention in the future. Although the successful of homogeneous catalyst has been investigated by many researchers, the limitation of this type of catalyst is their sensitivity to the existence of FFA. Due to this, development using heterogeneous catalyst in esterification process is one of the possible areas to be explored with the food waste feedstock. The bifunctional solids and acids that occur in heterogeneous catalyst were reported to have potential in catalyzing both free fatty acids esterification reaction and triglycerides transesterification at the same time.

It is also suggested that besides food waste, research could focused on different type of waste to be used as feedstock for biodiesel such as agricultural waste. Combining the two sources of waste as feedstock for biodiesel could be interesting area to study. It could not only provide alternative for current waste treatment method available in the country but also generating income in the future.

Thus, advanced understanding both in the types of organic feedstocks and synthesis pathway of fatty acid methyl ester could be a great future interest towards a more sustainable and environmentally biodiesel process.

REFERENCES

Aalok, A., Tripathi, A.K., Soni, P. (2008). Vermicomposting: a better option for organic solid waste management. *Journal of Human Ecology, 24 (1),* 59-64.

Abdul Hamid, A., Ahmad, A., Ibrahim, M.H., & Nik Abdul Rahman, N.H. (2012). Food waste management in Malaysia-current situation and future management options. *Journal of Industrial Research & Technology, 2(1),* 36-39.

Abdul Jalil, M. (2010). Sustainable development in Malaysia: a case study on household waste management. *Journal of Sustainable Development, 3 (3),* 91-104.

Agamuthu, P. (2001). *Solid waste principle and management with Malaysia case studies.* University Malaya Press, Kuala Lumpur, Malaysia.

Alam, M. M., & Rahman, K. A. (2013). Biodiesel from Mustard oil: a sustainable engine fuel substitute for Bangladesh. *International Journal of Renewable Energy Development (IJRED), 2(3),* 141-149.

Amankwah, E., & Darimani, H.S. (2012). Evaluation of biogas production and slurry from organic solid wastes for agricultural productivity and rural development. *International Journal of Current Research, 4 (2),* 111-116.

Amin, A., Abraham, C., Hamza, A. A., Abdalla, Z. A., Al-Shamsi, S. B., Harethi, S. S., & Daoud, S. (2012). A standardized extract of Ginkgo biloba neutralizes cisplatin-mediated reproductive toxicity in rats. *BioMed Research International*, *2012*.

Amthor, J., & Tan, D. (2013). *Bioenergy*. InTech Publishers.

Apte, A., Cheernam, V., Kamat, M., Kamat, S., Kashikar, P., & Jeswani, H. (2013). Potential of using kitchen waste in a biogas plant. *International Journal of Environmental Science and Development, 4(4),* 370-374.

Awolu, O.O., & Layokun, S.K. (2013). Optimization of two-step transesterification production from neem (Azadirachtaindica) oil. *International Journal of Energy and Environmental Engineering, 4(39),* 1-9.

Babalola, T. O. O., & Apata, D. F. (2011). Chemical and quality evaluation of some alternative lipid sources for aqua feed production. *Agriculture and Biology Journal of North America, 2(6),* 935-943.

Bada, J. C., León-Camacho, M., Prieto, M., & Alonso, L. (2004). Characterization of oils of hazelnuts from Asturias, Spain. *European Journal of Lipid Science and Technology, 106(5),* 294-300.

Bagudo, B.U., Garba, B., Dangoggo, S.M., & Hassan, L.G. (2011). The qualitative evaluation of biogas samples generated from selected organic wastes. *Archives of Applied Science Research, 3(5),* 549-555.

Bernal, J., Martín, M.T., & Toribio, L. (2013). Supercritical fluid chromatography in food analysis. *Journal of Chromatography A, 1313,* 24-36.

Bokhari, A., Yusup, S., & Ahmad, M. M. (2010). Optimization of the parameters that affects the solvent extraction of crude rubber seed oil using response surface methodology (RSM). *Recent Advances in Engineering*, 28-33.

Bolzonelle, D., Fatone, F., Pavan, P., & Cecchi, F. (2005). Anaerobic fermentation of organic municipal solid wastes for the production of soluble organic compounds. *Industrial& Engineering Chemistry Research, 44*, 3412-3418.

Boucher, M.B., Weed, C., Leadbeater, N.E., Wilhite, B. A., Stuart J., & Parnas, R.S. (2009). Pilot scale two-phase continuous flow biodiesel production via laminar flow reactor-separator. *Energy & Fuels, 23*, 2750-2756.

Budhiarta, I., Siwar, C., & Basri, H. (2012). Current status of municipal solid waste generation in Malaysia. *International Journal on Advanced Science, Engineering and Information Technology, 2(2),* 16-21.

Cao, Y., Liu, W., Xu, X., Zhang, H., Wang, J., & Xian, M. (2014). Production of free monounsaturated fatty acids by metabolically engineered Escherichia coli. *Biotechnology for Biofuels, 7(59),* 2-11.

Cardoso, A. L., Neves, S. C. G., & Da Silva, M. J. (2008). Esterification of oleic acid for biodiesel production catalyzed by SnCl2: a kinetic investigation. *Energies, 1(2),* 79-92.

Cataluna, R., & Silva, R. (2012).Effect of cetane number on specific fuel consumption and particulate matter and unburned hydrocarbon emission from diesel engines. *Journal of Combustion,* 1-6.

Chai, M., Tu, Q., Lu, M., & Yang, Y. J. (2014). Esterification pretreatment of free fatty acid in biodiesel production, from laboratory to industry. *Fuel Processing Technology, 125,* 106-113.

Chang, H.N., Kim, N., Kang, J., & Jeong, C.M. (2010). Biomass-derived volatile fatty acid platform for fuels and chemicals. *Biotechnology and Bioprocess Engineering, 15,* 1-10.

Chhetri, A.B., Tango, M.S., Budge, S.M., Watts, K.C., & Islam, M.R. (2008). Non-edible oils as new sources for biodiesel production. *International Journal of Molecular Sciences, 9,* 169-180.

Cho, J.K., Park, S.C., & Chang, H.N. (1995). Biochemical methane potential and solid state anaerobic digestion of Korean food waste. *Bioresource Technology, 52(3),* 245-253.

Chua, K. H., Yip, C. H., & Nie, W. L. S. (2008). A case study on the anaerobic treatment of food waste and gas formation. *Int. Conf. Constr. Build. Tech., F-29,* (pp. 311-316).

Dholakiya, B.Z. (2012). Superphosphoric acid catalyzed biodiesel production from low cost food stock. *Archives of Applied Science Research, 4(1),* 551-561.

Duhan, J.S., Kumar, A., & Tanwar, S.K. (2013). Bioethanol production from starchy part of tuberous plant (potato) using Saccharomyces cerevisiaeMTCC-170. *African Journal of Microbiology Research, 7(46),* 5253-5260.

Eddine, B. T., & Salah, M. M. (2012). Solid waste as renewable source of energy: current and future possibility in Algeria. *International Journal of Energy and Environmental Engineering, 3(1),* 1-12.

Ejikeme, P. M., Anyaogu, I. D., Ejikeme, C. L., Nwafor, N. P., Egbuonu, C. A. C., Ukogu, K., & Ibemesi, J. A. (2010). Catalysis in biodiesel production by transesterification processes-an insight. *Journal of Chemistry, 7(4),* 1120-1132.

Emmanuel, J. (2007). Best environmental practices and alternative technologies for medical waste management. *In Eighth International Waste Management Congress and Exhibition. Institute of Waste Management, Botswana, South Africa* (pp. 25-28).

Fakhry, E.M., Maghraby, D.M.E. (2013). Fatty acids composition and biodiesel characterization of Dunaliellasalina. *Journal of Water Resource & Protection, 5,* 894-899.

Fernández, A., Sánchez, A., & Font, X. (2005). Anaerobic co-digestion of a stimulated organic fraction of municipal solid wastes and fats of animal and vegetable origin. *Biochemical Engineering Journal, 26,* 22-28.

Gill, S.S., Jana, A.M., & Shrivastav, A. (2014). Anaerobic bacterial degradation of kitchen waste-a review. *International Journal of Research and Development in Pharmacy and Life Science, 3(2),* 850-854.

Ghosh, P., & Thakur, I. S. (2014). Enhanced removal of COD and color from landfill leachate in a sequential bioreactor. *Bioresource technology, 170,* 10-19.

Gnanaprakasam, A., Sivakumar, V.M., Surendhar, A., Thirumarimurugan, M., & Kannadasan, T. (2013). Recent strategy of biodiesel production from waste cooking oil and process influencing parameters: a review. *Journal of Energy,* 1-10.

Gravalos, I., Gialamas, T., Koutsofitis, Z., Kateris, D., Tsiropoulos, Z., Xyradakis, P., & Georgiades, A. (2008). Energetic study on animal fats and vegetable oils using combustion bomb calorimeter. *Journal of Agricultural Machinery Science, 4,* 69-74.

Hafid, H. S., Rahman, N. A. A., Omar, F. N., Yee, P. L., Abd-Aziz, S., & Hassan, M. A. (2010). A comparative study of organic acids production from kitchen wastes and simulated kitchen waste. *Australian Journal of Basic and Applied Sciences, 4(4),* 639-645.

Hashim, K. S. H. Y., Mohamed, A. H., & Redza, H. Z. M. S. (2012). Developing a waste minimization awareness model through community based movement: A case study of the IIUM Green Team. *Malaysia Journal of Society and Space, (5),* 112-123.

Hesnawi, R.M., & Mohamed, R.A. (2013). Effect of organic waste source on methane production during thermophilic digestion process. *International Journal of Environment Science & Development,* 435-437.

Hossain, A.B.M.S., Boyce, A.N., Salleh, A., & Chandran, S. (2010). Impacts of alcohol type, ratio and stirring time on the biodiesel production from waste canola oil. *African Journal of Agricultural Research, 5(14),* 1851-1859.

Hung, Y. S., Chen, Y. H., Shang, N. C., Chang, C. H., Lu, T. L., Chang, C. Y., & Shie, J. L. (2010). Comparison of biodiesels produced from waste and virgin vegetable oils. *Sustainable Environment Research, 20(6),* 417-422.

Ibeto, C. N., Okoye, C. O. B., & Ofoefule, A. U. (2012). Comparative study of the physicochemical characterization of some oils as potential feedstock for biodiesel production. *International Scholarly Research Notices*, 2012.

Ilham, Z., & Saka, S. (2011). Production of biodiesel with glycerol carbonate by non-catalytic supercritical dimethyl carbonate. *Lipid Technology, 23 (1),* 10-13.

Jain, S., Sharma, M. P., & Rajvanshi, S. (2011). Acid base catalyzed transesterification kinetics of waste cooking oil. *Fuel Processing Technology, 92(1),* 32-38.

Jaafar, M. N. M., Eldrainy, Y. A., & Asril, M. H. (2011). Experimental investigation of spray characteristics of refined bleached and deodorized palm oil and diesel blends using phase Doppler particle analyzer. *International Journal of Physical Sciences*, *6*(29), 6674-6680.

Johari, A., Ahmed, S. I., Hashim, H., Alkali, H., & Ramli, M. (2012). Economic and environmental benefits of landfill gas from municipal solid waste in Malaysia. *Renewable and Sustainable Energy Reviews, 16(5),* 2907-2912.

Jones, J., Manny, S., Montoya, M., Keller, K., Poenie, M. (2012). Extraction of Algal Lipids and their analysis by HPLC and mass spectrometry. *Journal of American Oil Chemistry Society, 89,* 1371-1381.

Kalanatarifard, A., & Yang, G. S. (2012).Identification of the municipal solid waste characteristics and potential of plastic recovery at Bakri Landfill, Muar, Malaysia. *Journal of Sustainable Development, 5(7),* 11-15.

Kargbo, D. M. (2010). Biodiesel production from municipal sewage sludges. *Energy & Fuels, 24(5),* 2791-2794.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T., & Dawson, L. (2011). The anaerobic digestion of solid organic waste. *Waste Management, 31,* 1737-1744.

Kim, J., Park, C., Kim, T.H., Lee, M., Kim, S., Kim, S.W., & Lee, J. (2003). Effects of various pretreatment for enhanced anaerobic digestion with waste activated sludge. *Journal of Bioscience and Bioengineering, 95(3),* 271-275.

Kim, S. S., Heo, H.S., Kin, S. G., Ryoo, R., Kin, J., Jeon, J.K., & Park, Y.K. (2011). Catalytic pyrolysis of oil fractions separated from food waste leachate over nanoporous acid catalysts. *Journal of Nanoscience and Nanotechnology, 11(7),* 6167-6171.

Kombe, G.G, Temu, A.K., Rajabu, H.M., Mrema, G.D., Kansedo, J., Lee, K.T. (2013). Pre-treatment of high FFAs oils by chemical re-esterification for biodiesel production – a review. *Advances in Chemical Engineering and Sciences, 3,* 242-247.

Kondusamy, D., &Kalamdhad, A. S. (2014). Pre-treatment and anaerobic digestion of food waste for high rate methane production–a review. *Journal of Environmental Chemical Engineering, 2(3),* 1821-1830.

Kraai, G. N., Winkelman, J. G. M., De Vries, J. G., & Heeres, H. J. (2008). Kinetic studies on the Rhizomucormiehei lipase catalyzed esterification reaction of oleic acid with 1-butanol in a biphasic system. *Biochemical Engineering Journal, 41(1),* 87-94.

Kusdiana, D., & Saka, S. (2004). Two-step preparation for catalyst-free biodiesel fuel production hydrolysis and methyl esterification. *Applied Biochemistry and Biotechnology, 113 (116),* 781-791.

Li, Y., Park, S.Y., Zhu, J. (2011). Solid-state anaerobic digestion for methane production from organic waste. *Renewable and Sustainable Energy Review,* 821-826.

Majlessi, M., Eslami, A., Saleh, H.N., Mirshafieean, S., Babaii, S. (2012). Vermicomposting of food waste: assessing the stability and maturity. *Journal of Environmental Health Science & Engineering, 9 (25),* 1-6.

Marchetti, J. M., Miguel, V. U., & Errazu, A. F., (2007). Possible methods for biodiesel production. *Renewable and Sustainable Energy, 1 (1),* 1 300-1 31.

Mathiyazhagan, M., & Ganapathi, A. (2011). Factor affecting biodiesel production. *Research in Plant Biology, 1 (2),* 1-5.

Mishra, S.R., Mohanty, M.K., Das, S.P., & Pattanaik, A.K. (2013). Optimisation of base-catalysed transesterification of Simaroubaglauca oil for biodiesel production. *International Journal of Sustainable Energy,* 1-8.

Misra, R. D., & Murthy, M. S. (2010). Straight vegetable oils usage in a compression ignition engine—a review. *Renewable and Sustainable Energy Reviews, 14(9),* 3005-3013.

Mohamed Ali, A.S., & Abdurrhman, A.M. (2013). Determination of free fatty acids in palm oil samples by non-aqueous flow injection using salicyaldehyde-2, 4-dinitrophenylhydrazone as colometric reagent. *Chemical and Material Engineering, 1 (3),* 96-103.

MONSAL. (2011). March food waste anaerobic digestion plant: construction underway. Retrieved July 2, 2012, from http://blog.monsal.com/index.php/news/march-food-waste-anaerobic-digestion-plant-construction-underway/.

Montes, J. M., Rodríguez Aliciardi, M. J., Vaca Chávez, J. C., Guzmán, C., & Calandri, E. (2011). Characterization of Jatrophacurcas L. seed and its oil, from Argentina and Paraguay. *The Journal of the Argentine Chemical Society, 98,* 1-9.

Moukamnerd, C., Kawahara, H., & Katakura, Y. (2013). Feasibility Study of Ethanol Production from Food Wastes by Consolidated Continuous Solid-State Fermentation.

Nakpong, P., & Wootthikanokkhan, S. (2009). Biodiesel production from mixtures of vegetable oil and used cooking oil. *Asian Journal on Energy and Environment, 10 (4),* 221-229.

Nakpong, P., & Wootthikanokkhan, S. (2010). Mixtures of vegetable oil and used frying oil as feedstock for biodiesel production in Thailand. *Suranaree Journal of Science and Technology (Thailand).*

Ogbu, I.M., & Ajiwe., V.I.E. (2013). Biodiesel production via esterification of free fatty acids from Cucurbitapepo L. seed oil : kinetic studies. *International Journal of Science and Technology, 2 (8),* 616-621.

Oh, S.T., & Martin, A.D. (2010). Long chain fatty acids degradation in anaerobic digester : thermodynamic equilibrium consideration. *Process Biochemistry, 45, 335-345.*

Pagans, E., Font, X., & Sánchez, A. (2006). Emission of volatile organic compounds from composting of different solid waste: abatement by biofiltration. *Journal of Hazardous Materials B131,* 179-186.

Patil, P.D., Gude, V. G., Mannarswamy, A., Cooke, P., Nirmalakhandan, N., Lammers, P., & Deng, S. (2012). Comparison of direct transesterification of algal biomass under supercritical methanol and microwave irradiation condition. *Fuel, 97,* 822-831.

Pleissner, D., & Lin, C.S.K. (2013).Valorisation of food waste in biotechnological processes. *Sustainable Chemical Processes, 1(21),* 1-6.

Rao, M. S., & Singh, S. P. (2004). Bioenergy conversion studies of organic fraction of MSW: kinetic studies and gas yield–organic loading relationships for process optimisation. *Bioresource Technology, 95(2),* 173-185.

Rashad, M., & Shalaby, E. A. (2007). Dispersion and deposition of heavy metals around two municipal solid waste (MSW) dumpsites, Alexandria, Egypt. Am. Eurasian *Journal of Agricultural and Environmental Science, 2(3),* 204-212.

Redzwan, G., & Banks, C.J. (2010). Supplementation of ammonium bicarbonates for the treatment of fruit cordial wastewater by anaerobic digestion process. *Anaerobe, 16 (1),* 34-37. doi:10.1016/j.anaerobe.2009.04.00.

Romano, S. D., & Sorichetti, P. A. (2011). *Electric Properties of Liquids. In Dielectric Spectroscopy in Biodiesel Production and Characterization.* Springer London (pp. 43-57).

Rozainee, M., Ngo, S. P., Salema, A. A., Tan, K. G., Ariffin, M., & Zainura, Z. N. (2008). Effect of fluidising velocity on the combustion of rice husk in a bench-scale fluidised bed combustor for the production of amorphous rice husk ash. *Bioresource Technology, 99(4),* 703-713.

Sahu, O. (2014). Bioethanol production by coffee husk for rural area. *Advanced Research Journal of Biochemistry and Biotechnology, 1 (1),* 001-005.

Saleh, G., & Rajanaidu, N. (2011). Directions in the utilization of crops for bio-energy in Malaysia in response to climate change. *Journal of ISSAAS (International Society for Southeast Asian Agricultural Sciences), 17(2),* 1-13.

Samsudin, M. D. M., & Don, M. M. (2013). Municipal solid waste management in Malaysia: current practices, challenges and prospects. *Jurnal Teknologi, 62(1).*

Sánchez, A., Maceiras, R., Cancela, A., & Rodríguez, M. (2012). Influence of n-hexane on in situ transesterification of marine macroalgae. *Energies, 5,* 243-257.

Sani, Y. M., Daud, W. M. A. W., & Aziz, A. A. (2013). Solid acid-catalyzed biodiesel production from microalgal oil—the dual advantage. *Journal of Environmental Chemical Engineering, 1(3),* 113-121.

Scrimgeour, C. (2005). *Bailey's industrial oil and fat products.* John Wiley & Sons, Inc.

Shalaby, E. A., & El-Gendy, N. S. (2012). Two steps alkaline transesterification of waste cooking oil and quality assessment of produced biodiesel. *International Journal Chemistry Biochemical Science, 1(3).*

Silva, F.C., Serafim, L., Nadais, H., Arroja, L., & Capela, I. (2013). Acidogenic fermentation towards valorisation of organic waste streams into volatile fatty acids. *Chemical and Biochemical Engineering Quarterly, 27 (4),* 467-476.

Sims, R.E., Mabee, W., Saddler, J.N., & Taylor, M. (2010). An overview of second generation biofuel technologies. *Bioresource technology, 101(6),* 1570-1580.

Singh, S.P., & Singh, D. (2010). Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel : a review. *Renewable and Sustainable Energy Reviews, 14,* 200-216.

Singh, R., Tiwari, S., Srivastava, M., & Shukla, A. (2014). Experimental study on the performance of microwave assisted Hydrogen peroxide (H202) pretreatment of rice straw. *Agricultural Engineering International: CIGR Journal, 16(1),* 173-181.

Skoog, D. A., West, D. M., Holler, F. J., & Crouch, S. (1999). *Analytical chemistry, an introduction,* Thomson Learning. Inc, USA.

Sreenivasan, J., Govindan, M., Chinnasami, M., & Kadiresu, I. (2012). *Solid waste management in Malaysia: a move towards sustainability. Waste management: an integrated vision.* In Tech, pp 348.

Stoeberl, M., Werkmeister, R., Faulstich, M., & Russ, W. (2011). Biobutanol from food wastes-fermentative production, use as biofuel and the influence on the emission. *Procedia Food Science, 1,* 1867-1874.

Subramani, T., & Ponkumar, S. (2012). Anaerobic digestion of aerobic pretreated organic waste. *International Journal of Modern Engineering Research, 2(3),* 607-611.

Syed Ismail, S.N., & Abd Manaf, L. (2013). The challenge of future landfill: a case study of Malaysia. *Journal of Toxicology and Environmental Health Sciences, 5 (6),* 86-96.

SYSAV Biotech AB. (2012). From food waste to new resources.SYSAV's pre-treatment plant for food waste. http://www.sysav.se.(Accessed September 2012).

Talebian-Kiakalaieh, A., Amin, N. A. S., Zarei, A., & Jaliliannosrati, H. (2013). Biodiesel production from high free fatty acid waste cooking oil by solid acid catalyst. *In Proceedings of the 6th International Conference on Process Systems Engineering (PSE ASIA), 25,* p. 27.

The World Bank. (2007). People with Disabilities in India: From Commitment to Outcomes. May2007. http://siteresources.worldbank.org/INDIAEXTN/Resources/295583-1171456325808/DISABILITYREPORTFINALNOV2007.pdf.

Uddin, G. S., Tiwari, A. K., Arouri, M., & Teulon, F. (2013). On the relationship between oil price and exchange rates: A wavelet analysis. *Economic Modelling, 35,* 502-507.

Viele, E. L., Chukwuma, F. O., & Uyigue, L. (2013). Esterification of high free fatty acid crude palm kernel oil as feedstock for base-catalyzed transesterification reaction. *International Journal of Application or Innovation in Engineering & Management (IJAIEM), 2(12),* 361-365.

Vikrant, D., & Shekhar, P. (2013). Generation of biogas from kitchen waste-experimental analysis. *International Journal of Engineering Science Invention, 2 (10),* 15-19.

Vintila, T., Gherman, V., Bura, M., Dragomirescu, M., Ilie, D., Julean, C., & Neo, S. (2013). Biogas generation from corn stalks and corn stalks bagasse resulted from ethanol production. *Romanian Biotechnological Letters, 18 (6),* 8812-8822.

Wanasolo, W., Manyele, S.V., & Makunza, J. (2013). A kinetic study of anaerobic biodegradation of food and fruit residues during biogas generation using initial rate method. *Engineering, 5,* 577-586.

Wang, L., & Weller, C.L. (2006). Recent advances in extraction of nutra-ceuticals from plants. *Trends in Food Science & Technology, 17,* 300-312.

Yadav, A., Gupta, R., & Grag, V.K. (2013). Organic manure production from cow dung and biogas slurry by vermicomposting under field conditions. *International Journal of Recycling of Organic Waste in Agriculture, 2 (21),* 1-7.

Yahaya, N., & Larsen, I. (2008). Federalising solid waste management in peninsular Malaysia. *In Proceeding of International Solid Waste Association (ISWA) World Congress, Singapore*.

Yu, M.J., Jo, Y., Kim, S., Lim, Y., Jem, J., Park, S.H., Kim, S., Park, Y. (2011). Synthesis of biodiesel from an oil fraction separated food waste leachate. *Korean Journal of Chemical Engineering, 28 (12),* 2287-2292.

LIST OF PAPER PRESENTED

Amin, M.M., Ilham, Z., & Redzwan, G. (2014). Utilization of volatile fatty acids (VFAs) from food waste leachate as feedstock for biodiesel synthesis. *In Proceeding of The 3R International Scientific Conference on Material Cycles and Waste Management, Japan.*

Redzwan, G, Amin, M.M., Zulkarnain, N.N., Mansor, M.R.A., Annuar, M.S.M., & Ilham, Z. (2014). Extrication of biodiesel feedstock from early stage of food waste liquefaction. *Journal of Material Cycles and Waste Management.* Under review.