MICROWAVE ASSISTED EXTRACTION METHOD FOR LIPID EXTRACTION FROM FOOD WASTE

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ABSTRAK

Jumlah sisa makanan yang banyak telah mengakibatkan masalah pengendalian dan pelupusan. yang telah membahayakan kesihatan manusia dan mencemarkan alam sekitar. Salah satu langkah yang paling penting untuk menyelesaikan masalah sisa makanan ialah menggunakan semula sisa makanan untuk pengeluaran biodiesel di samping memenuhi permintaan tenaga. Objektif projek ini adalah untuk menentukan keadaan optimum pengekstrakan lipid daripada mikroalga sisa makanan dengan menggunakan kaedah pengekstrakan dibantu gelombang mikro (MAE) dan melakukan analisis ke atas komposisi asid lemak bebas (FFA) dalam lipid mikroalga dengan menggunakan kromatografi gas-spektrometri jisim (GC-MS). Sisa makanan yang digunakan terdiri daripada karbohidrat dan protein yang sesuai untuk pertumbuhan mikroalga. Ia dikisar sebelum dibiakkan dalam botol jernih di bawah cahaya matahari selama sebulan. Kemudian, ia dikeringkan dengan menggunakan ketuhar pada suhu 105 ℃. Pelarut metanol digunakan untuk pengekstrakan lipid. Parameter yang dikaji pada pengekstrakan lipid termasuk kesan masa tindak balas ((10, 15, 20, 25, 30) min) dan kesan jumlah pelarut ((300, 350, 400, 450, 500) ml). Hasilnya, lipid diekstrak dalam keadaan optimum dengan hasil 95 %. Ia juga diperhatikan bahawa panjang rantai karbon untuk FFA dikenal pasti kebanyakannya dalam C16-C18 yang disyorkan untuk pengeluaran biodiesel.

ABSTRACT

Large amount of food waste has resulted in handling and disposal problem, which have harmed human health and polluted the environment. One of the most important measures to solve food waste problem is to reuse food waste for biodiesel production while also fulfilling energy demand. The objectives of this project are to determine the optimum condition of lipid extraction from food waste microalgae by using microwave assisted extraction (MAE) method and to perform analysis on free fatty acid (FFA) composition in microalgae lipid by using gas chromatography-mass spectrometry (GC-MS). The food waste used consist of carbohydrate and protein which are suitable condition for microalgae growth. It was grinded before being cultivated in a clear bottle under sunlight for a month. Then, it was dried by using oven at 105 ℃. Methanol solvent was used for lipid extraction. The parameters studied on lipid extraction include effect of reaction time ((10, 15, 20, 25, 30) min) and effect of solvent amount ((300, 350, 400, 450, 500) ml). As a result, lipid was extracted under optimum condition with yield of 95 %. It was also observed that the carbon chain length for FFA identified mostly within C16-C18 which are recommended for biodiesel production.

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℃	Degree Celsius
%	Percentage

LIST OF ABBREVIATIONS

CO_2	Carbon dioxide
SO_2	Sulphur dioxide
PM	Particulate matter
OLR	Outgoing longwave radiation
CO	Carbon monoxide
FW	Food waste
MAE	Microwave assisted extraction
FFA	Free fatty acid
GC-MS	Gas chromatograph mass spectrophotometer
FAME	Fatty acid methyl esters
GC-FID	Gas chromatograph flame ionization detector
ASTM D6751	American Society for Testing and Materials
US	United State
pH	Potential of hydrogen
MUFAs	Mono-unsaturated fatty acids
PUFAs	Polyunsaturated fatty acids

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nowadays, globalization has been rapidly evolving and become dominant in many countries. As the number of developed countries increases, the demand for fossil fuels also increase. Fossil fuel is one of the important sources of energy production. The develop countries need large amount of energy especially for their electricity and transportation sectors (Amin and Prabandono, 2017). It is about 40.5 % of the total energy have been used for transportation sectors (Hamza *et al*., 2021). Energy is produced by combustion of the fossil fuels before converted to electricity. Fossil fuels are including coal, oil and natural gas which are come from organic material over the course of millions of years and have fuelled the world over the past century. In the period of 1995 until 2015, the consumption of fossil fuel increased approximately 51 % and it is predicted that the consumption will increase approximately 18 % more in the period of 2015 until 2035 (Yildiz, 2018).

However, the main drawback of it, is environmental pollution. This pollution occurs because of carbon dioxide (CO_2) and pollutants such as particulate matter (PM) and sulphur dioxide (SO_2) are released to the environment during the fossil fuels combustion (Shi *et al*., 2021). The emission of CO_2 is around 21.3 billion tonnes per year (Yildiz, 2018). CO_2 is a greenhouse gas, which reduce the amount of outgoing longwave radiation (OLR) to space therefore contributes to global warming. This gas not only contributes to global warming but also affect aspects in human life (Kalsum *et al*., 2018). The other condition such as incomplete fossil fuels combustion produces carbon monoxide (CO) which is a very harmful and poisonous gas. Inhalation of this gas is likely to cause death because it interferes oxygen transportation in the human blood stream.

Apart from that, the world needs alternative fuels that offer a harmonious relationship with sustainable growth, energy conservation, good quality and environmental protection. The best alternative fuel is biodiesel. Biodiesel comes in three types of generations, first, second, and third generations of biodiesel. They are distinguished by their biomass sources, limits as a renewable energy source, and technological advancement. First generation biodiesel feedstock is derived from food and edible oils such as soybean and oil palm (Mofijur *et al*., 2020). For second generation biodiesel feedstock, it is obtained from non-edible sources such as wood and husk. The second-generation biodiesel is more environmentally friendly compared to the first-generation biodiesel because it does not reduce the amount of edible food for human consumption. However, it still has problems. This is because the cultivation of non-edible crops for second generation biodiesel requires an extensive amount of fertile land, which competes with land used for the cultivation of edible food crops.

While third generation biodiesel feedstock are derived from microorganisms which are photosynthetic such as microalgae (Wood, 2021). This alternative fuels are not affecting both the food and land problems related to first- and second-generation biodiesel. Therefore, it is recommended to use third generation biodiesel as a substitute for fossil fuels because they are sustainable, renewable and eco-friendly compare to feedstock used for first- and second-generation biodiesel (Jacob *et al*., 2021). Most of researches have been identify the best alternative way of reusing renewable resources-based microalgae especially from waste product for biodiesel production. Waste products like food waste are zero cost raw materials that can be used for biodiesel production which also will help to minimize waste generation and disposal problem (Barik *et al*., 2018).

1.2 Problem Statement

Rapid urbanization and industrialization result in a large amount of food waste (FW) which resulting in handling and disposal problem. Every day, several billion gallons of FW generated from hostels, restaurants, kitchens of residential societies, and food and meat processing industries are collected and thrown in an open area or dumped in landfill without proper disposal technique. These problems can affect human health and polluting environment because it produces harmful leachate when rainwater falls on it. In the above context, reuse of FW for biodiesel production is one of the key steps to reduce FW problem and also meet the energy demand (Barik *et al*., 2018). This is because lipid contents in FW microalgae can be converted into biodiesel. The lipid are vary widely, reaching up to 80 % in some cases (Jafari *et al*., 2021). However, isolation of lipid is required since FW contains other substances such as carbohydrates, lipids, phosphates, vitamins and amino acids (Karmee, 2016). Therefore, lipid extraction is considered as an important step to produce biodiesel from FW microalgae. The yield of biodiesel production depends on composition of fatty acid in lipid molecule of FW (Yusuff *et al*., 2021).

To carry out extraction process, disruption of microalgae cell wall is necessary. Microalgae are made up of highly complex cell walls which is not easy to break and extract the lipid completely. The selection of lipid extraction method is depending on the algae cell type primarily to use either both non-polar and/or polar solvent (Vasistha *et al*., 2021). There is few research have been done on lipid extraction from various types of sources, but still no research yet reporting on the suitable solvent polarity of lipid recovery for biodiesel production from FW microalgae (Yin *et al*., 2020). In this study, knowledge about the suitable polarity of solvent for lipid recovery from FW microalgae is needed. This is because this knowledge allows lipid extraction to occur at optimum condition if suitable solvent is used.

1.3 Objectives

There are two objectives from this project which are:

i. To determine optimum condition of lipid extraction from food waste microalgae under effect of solvent amount and effect of reaction time by using microwave assisted extraction (MAE) method.

ii. To perform analysis on free fatty acid (FFA) composition in food waste microalgae lipid.

1.4 Scope of the study

The study focuses on the production of biodiesel from microalgae derived from food waste (FW). The FW were collected from kitchen waste at home. Type of FW that were used to cultivate the microalgae includes carbohydrates and proteins such as leftover rice, cake, fruits, bread, chicken, meat, fish and vegetables. The microalgae were cultivated for a month before harvested for lipid extraction. The lipid from the microalgae were extracted by microwave assisted extraction (MAE) method by using methanol solvent. The parameters studied on lipid extraction include effect of solvent amount ((300, 350, 400, 450, 500) ml) and effect of reaction time ((10, 15, 20, 25, 30) min) in order to obtain efficient yield of lipid extraction. The lipid content was further calculated and analysed by using gas chromatograph mass spectrophotometer (GC-MS) to determine the composition of free fatty acids (FFA).

1.5 Significant of Study

Energy generation is critical for societal development since it allows for economic and social progress. However, today's energy dependence on fossil fuels has resulted in a number of drawbacks, including the conventional energy's sustainability and the impact of carbon emissions from fossil fuels burning. The findings of this study will contribute to a possible alternative for fossil fuels that can address a variety of energy and environmental challenges by using food waste (FW) microalgae for biodiesel synthesis.

This study focused on microalgae as an energy source, which has been investigated as a potential solution to the limitations of first and second generation biofuels. Microalgae can be a potential answer to concerns such as land usage and competition between food crops and biofuels, as it does not require agricultural land and can be cultured in areas that are unsuitable for agriculture. As a result, microalgae farming is low-cost and can help to reduce the burden of agricultural land utilisation. Furthermore, because algal biodiesel emits little to no carbon, this study contributes to carbon neutrality.

Furthermore, this study encourages the use of food waste, which can help to minimise land and resource waste because food waste is typically produced in large quantities and requires a lot of space to dispose of. Food waste can also reduce the danger of environmental pollution and health risks associated with traditional disposal methods such as combustion and dumping in open areas. This research also encourages the decrease of food waste's carbon emissions, which has the potential to generate harmful gasses like greenhouse gases and other pollutant gases. As a result, this study indicated the future potential of renewable energy, which may be less dangerous to the environment.

CHAPTER 2

LITERATURE REVIEW

2.1 Biodiesel

Biodiesel has been regarded as one of the most promising renewable fuels. This is because of its biodegradability, resilience, and role in reducing pollutant emissions in recent years (Naylor and Higgins, 2017). Biodiesel is produced in many countries around the world from a variety of sources. Furthermore, due to the advent of subsidies and tax exemptions, biodiesel has become more economical and is now widely used in many parts of the world. Biodiesel is a long-chain ester (C14-C24) that can be made from a variety of lipid sources, including palm oils, animal fats, and waste oil *(*Khoobbakht *et al*., 2016).

2.1.1 Biodiesel Generation

Biodiesel is a combination of mono-alkyl esters of long-chain fatty acids which known as fatty acid methyl esters (FAME) made from a variety of organic lipid feedstock and biomass (Behera *et al*., 2015). Biodiesel is commonly classified as first, second, and third generation based on its source. Over 350 oil-bearing plants have been identified as potential sources of biodiesel, which can be classified into three generations: first, second, and third (Mofijur *et al*., 2020).

2.1.1.1 First Generation

Crop and edible oils are first-generation biodiesel feedstock. Soybean, sunflower, oil palm, rapeseed, canola, and cottonseed are some of the most popular feedstock for first-generation biodiesel (Samani *et al*., 2021). However, it has been proposed that using edible food crops for the manufacture of first-generation biofuels decreases the amount of edible food available for human consumption, thus raising global food prices (Bhuiya *et al*., 2020). Although first generation biofuels help satisfy the human need for fuel, at

the same time it depletes some resources intended for the even more important human need for nourishment. This provides an incentive for researchers to explore other sources of biofuels that do not disrupt the human food supply.

2.1.1.2 Second Generation

Second-generation biodiesel is made from non-edible feedstock such as crops, non-edible oil, and other non-edible sources such as wood, husk, and other non-edible products, which are then refined to make biodiesel (Rahman *et al*., 2016). These sources completely eliminate us rely on edible food crops for fuel supply, which is what started the "food vs. fuel" controversy in the first place. Jatropha, mahua, jojoba oil, tobacco seed, Calophyllum, and sea mango are some of the feedstock used to make second generation biodiesel (Lee *et al*., 2020). Commercial and residential waste is also included in this category.

As opposed to the feedstock used to make first generation biodiesel, using this feedstock to make second generation biodiesel is more environmentally sustainable. This is due to the fact that growing this feedstock requires no extra fertiliser, water, or soil. However, there are still some issues. Crops need fertile land to grow by their own existence, and the planting of non-edible crops for second generation biodiesel necessitates a large amount of fertile land, which competes with land used to grow edible food crops.

2.1.1.3 Third Generation

Third-generation biodiesel solves the food and land issues that first and second generation biodiesel pose. Algae, specifically microalgae, is used as a feedstock for third generation biodiesel production (Chia *et al*., 2018). As compared to the feedstock used in first and second generation biodiesel, the use of microalgae for biodiesel production is thought to be a more viable choice (Saladini *et al.*, 2016). In terms of plantation area, microalgae have the ability to generate yields that are 15-300 times higher than conventional crops (Hossain *et al*., 2019).

2.1.2 Properties of Biodiesel

The concentration of fatty acid methyl ester in purified biodiesel was determined using a gas chromatograph flame ionization detector (GC-FID). Biodiesel properties were also compared to a variety of standards. The properties of biodiesel indicate whether or not it will be ideal for the engine's efficiency, life, and emissions. The acid number, calorific value, viscosity, density, flash point, fire point, cloud point, pour point, ash content, and carbon residue are all important properties of biodiesel (Karmakar *et al*., 2018).

The lipids are converted into fatty acid methyl ester (FAME) rich biodiesel precursors during the transesterification process (plus glycerol). However, the quality of these raw biodiesel precursors varies, and they may need to be upgraded and blended to meet engine-fuel grade biodiesel standards and other performance criteria. The low-temperature properties, storage safety, and related NOx emissions of marketable biodiesel products are also addressed (Wood, 2021).

The detailed fuel properties of biodiesel made from microalgae, as well as the related engine emissions characteristics, have been thoroughly investigated by (Karmakar *et al*., 2018). Biodiesel comprises between 10 % and 12 % oxygen, allowing it to fully combust with lower average exhaust emissions but slightly higher NOx emissions than fossil-fuel derived diesel fuel.

2.1.3 Quality Standard of Biodiesel

ASTM D6751 (American Society for Testing and Materials) specifies biodiesel requirements and test methods. ASTM D6751 specifies main biodiesel fuel properties, the first for the biodiesel blend portion and the second for both blend stock and neat biodiesel automotive fuel. Biodiesel is defined as mono-alkyl esters of long chain fatty acids derived from vegetable oils and animal fats, according to ASTM D6751, a US standard. The type of alcohol used is unspecified. Thus, any alcohol (methanol, ethanol, etc.) may be used to make mono-alkyl esters as long as it fulfilled the detailed specifications specified in the fuel specification. By requiring that the fuel be mono- alkyl esters of long chain fatty acids, other components, with the exception of additives, would

inherently be excluded. Biodiesel is made from a variety of alcohols, and the type of alcohol used in the process makes no difference chemically as long as the resulting biodiesel meets ASTM D6751 (Musa, 2016).

2.2 Microalgae

Protein, holocelluloses (carbohydrates found mostly in the cell wall, such as hemicelluloses and cellulose), lipids, and other components like as photosynthetic pigments make up the majority of microalgae (chlorophylls, carotenoids, and phycobilins) (Figueira *et al.*, 2015). Microalgae has recently received a lot of research as a potential alternative energy source (Jin *et al*., 2016). Microalgae are being considered as a viable biofuel feedstock by scientists, academics, and entrepreneurs in the biofuel industry due to their better biomass production, adaptability in growing in various sorts of environments, and greater oil or lipid content than land-based sources (Sharma *et al*., 2018). Lipid triggering conditions must be optimised and maintained in order to achieve increased lipid content from microalgae cultivated in the lab or outdoors.

Various parameters, including as light intensity, temperature, pH, and CO_2 concentration, may enhance lipid content or production. Physical characteristics of the medium, such as light, temperature, and nutritional supplements, impact not only the strain's metabolic machinery, but also the composition and production of microalgae lipids (Mathimani *et al*., 2018). Physical variables, as well as the medium composition, affect the lipid content of a specific algae species over its life cycle. Light-driven cell factories (algae) produce carbohydrates, lipids, and proteins during photosynthesis, which is dependent on a variety of parameters such as light intensity, temperature, CO_2, and pH levels (Brindhadevi *et al*., 2021).

2.3 Lipid Extraction

Lipid is extracted in the presence of solvents, enzymes, and, depending on the substance, mechanical pressing in the presence of solvents (with or without heating, or under supercritical or subcritical conditions). (Mathimani and Mallick, 2018). Lipids are insoluble in water, they can be separated from the proteins, carbohydrates, and water that make up the material to be extracted. Because lipids have such a wide range of relative

hydrophobicity, using a single universal solvent for lipid extraction is nearly impossible. Nonpolar solvents may extract neutral lipids, which are covalently linked, but polar lipids, which are bound by hydrogen and electrostatic forces, need polar solvents capable of breaking those bonds and releasing them (de Jesus and Filho, 2020).

Lipid extraction from microorganisms is done in the industrial setting following the drying or dehydration process (Patel *et al*., 2018). Microalgae pre-treatment for cell disruption and lipid extraction is an energy-intensive procedure that restricts the long-term viability of microalgae biofuel production. Microalgae have very complex cell walls containing intercalated polysaccharides and protein (To *et al*., 2018). Breaking the cell wall and extracting the lipid fully without a substantial quantity of energy is difficult. Depending on the algal cell type, an appropriate cell lysis procedure is usually done prior to lipid extraction. Mechanical (homogenizer, sonication, microwave, pulse electric field) and non-mechanical or chemical (acid, surfactant, enzymes) approaches are used to disturb cells (Vasistha *et al*., 2021).

Furthermore, there is a differential between chemical and mechanical approaches, with chemical methods being more scalable than mechanical ones (de Carvalho *et al*., 2020). Following cell disruption, lipid extraction is performed using polar (methanol, chloroform) and non-polar (hexane) solvents. Extraction of lipids using appropriate environmentally friendly solvents is a difficult aspect of biodiesel development. Alternative solvents that may be used for lipid extraction without harming the environment or human health are urgently needed. To obtain high efficiency and superior outcomes, one step integration technology integrating cell disruption and lipid extraction procedures may be applied.

2.3.1 Microwave Assisted Extraction (MAE)

Microwaves utilise high-frequency waves to create water molecule vibrations inside microalgae biomass, which raises the temperature and pressure caused by evaporation of water, causing cell wall breach (Kapoore *et al*., 2018). It's a sustainable method that allows for quick energy transmission as well as simultaneous heating of organic material and solvent assembly. These qualities increase extraction yield, decrease extraction time, and reduce the amount of solvent needed (Dahmoune *et al.*, 2021). When

compared with the conventional approach, the microwave uses around 23 times less energy. This is due to the fact that microwave energy is transferred directly to the reactant, removing the need for preheating. In addition, the MAE process has a more efficient heat transfer system than the conventional method. Microwave delivers energy in the form of electromagnetic waves, while the traditional approach sends heat to the process through thermal heat reflux (Tan *et al*., 2019). MAE has the following advantages: it is more cost effective, environmentally friendly, has a shorter extraction time, uses less solvent, and has a higher extraction yield (Mofijur *et al*., 2019). A variety of techniques may be used to effectively destroy microalgae cells. Microwaves, on the other hand, may be used to scale up lipid extraction to be efficient, environmentally friendly, and cost-effective in commercial-scale biodiesel operations (Wood, 2021). Microwaves have been favoured in recent studies and have long been acknowledged as providing better results.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter focus on microalgae cultivation, lipid extraction and analysis of free fatty acids (FFA) in lipid molecule in order to achieve the objectives of this study.

3.2 Lipid Extraction from Food Waste (FW) Microalgae

3.2.1 Material, Chemical and Reagent

The microalgae were obtained from the food waste (FW) which are collected from kitchen waste. There was 10 kg of FW had been cultivated which consist of carbohydrates and proteins such as leftover rice, cake, fruits, bread, chicken, meat, fish and vegetables. Solvent that was used for lipid extraction is methanol.

3.2.2 Microalgae Cultivation

The microalgae from the food waste (FW) was cultivated in a clear bottle under sunlight for a month. Before the cultivation process, the FW was grinded into small particles. Then, the moisture content was removed from the FW by oven drying at 105°C for 24 hours (Barik *et al*., 2018). The samples were further grinded into fine powder by using 240 V blender before proceed to lipid extraction method.

3.2.3 Lipid Extraction

The parameters studied for lipid extraction include effect of reaction time and effect of solvent amount in order to obtain efficient yield of lipid extraction. For effect of reaction time, 200 g of samples and 300 ml of methanol were mixed in 1 L round bottom flask. Then, the mixture was placed in a microwave extractor with power of 400 W at 60 °C for ((10, 15, 20, 25, 30) min). Next, for effect of solvent amount, 200 g sample was

mixed with ((300, 350, 400, 450, 500) ml of methanol in 1 L round bottom flask. Then, the mixture was placed in microwave extractor at 60 °C for 10 min.

After extraction, the mixture was centrifuged at 3000 rpm for 15 min before filter the extracted lipid (Zhou *et al*., 2019). Then, the extracted lipid was placed in conical flask then put into oven at 65 °C until reach a constant volume. Next, the percentage of total lipid extracted was calculated by using this equation (Ahmed *et al.*, 2019):

$$\text{Lipid content } \% = \frac{\text{Weight of oil content after extraction}}{\text{Weight of dry sample}} \times 100\ \% \qquad 3.1$$

Lastly, the lipid extraction was further analysed by using gas chromatograph mass spectrophotometer (GC-MS) to determine the composition of free fatty acid (FFA) present that will influence the production of biodiesel (Barik *et al*., 2018).

3.2.4 Identification of Free Fatty Acid (FFA) Composition through Gas Chromatography Mass Spectrometer (GC-MS)

The free fatty acids were identified by using gas chromatography mass spectrometer (GC-MS) with split automatic injector and capillary column. The operating condition for the GC-MS was 1 ml/min carrier gas flow of Helium (He) with column held at 150 °C for 1 minute and ramped to 240 °C at rate of 30 °C/minute. Then, the column was held at 240 °C for 30 minutes. The injector temperature was set to 250 °C and the detector was at 280 °C (Mansour *et al*., 2019).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

Microwave assisted extraction (MAE) method to extract lipid from food waste microalgae in this project was successfully achieved under two different condition which are effect of solvent amount and effect of reaction time. The extracted lipid could be run for free fatty acid (FFA) analysis by using gas chromatograph mass spectrophotometer (GC-MS) after lipid extraction process. The highest extracted lipid amount was chosen for each condition, so only 2 samples was analysed. The amount of extracted lipid used is 10 ml for GC-MS method. The analysis was to determine the effectiveness of food waste microalgae lipid to be used for biodiesel production based on the FFA composition.

Moreover, more preliminary study was needed prior to the lipid extraction for the optimum condition. The cultivation of food waste in 30 days was significantly essential and gave huge effect in the analysis process. The main parameters or variables that was considered and measured in this project were the amount of extracted lipid as well as FFA composition. The optimum amount of solvent and reaction time was the major key role to the effectiveness of lipid extraction. Other than that, The FFA composition also gave effect towards the efficiency for biodiesel production. However, not all fatty acids are suitable for biodiesel production, the recommendation from previous study was within 16 and 18 carbon length (Qiu *et al*., 2017).

The best results obtained for lipid extraction with respect to effect of solvent amount and effect of reaction time are (500 ml of solvent, 60 ℃, 200 g of sample, 10 min) and (300 ml of solvent, 60 ℃, 200 g sample, 25 min) respectively. Besides, fatty acid profile showed within carbon chain length C8-C18.

4.2 Microalgae Cultivation

Figure 4.1 Food waste cultivation on first day.

Figure 4.2 Food waste cultivation after a month.

Figure 4.2 showed the food waste after cultivation in a month. The appearance of microalgae on the top layer prove that the condition was suitable for microalgae growth.

4.3 Moisture Content

Removal of moisture content is a vital step in lipid yield because water present in sample may inhibit the solvent to penetrate inside the sample. In general, lesser the moisture content, more amount of lipid yield. The moisture of the sample is dependent on drying method and temperature. Oven drying method was selected to produce more lipid yield. This method have been conducted at 105 ℃ for 24 h. The optimized temperature for drying is 105 ℃, very high temperature in oven drying will burn the sample and break the fatty acid chain because for biodiesel production long chain fatty acids are required (Barik *et al*., 2018). The dried sample have been showed in Figure 4.1.

Figure 4.3 Dried food waste.

4.4 Lipid Analysis

Microwave assisted extraction was used to extract lipids using methanol as the solvent. Pre-treatment methods such as ultrasonic and microwave assisted techniques, in addition to solvent extraction, may improve lipids extraction (Jacob *et al*., 2021). Triglycerides, phospholipids, and sterols are the three major forms of lipids. Lipids are a broad group of nonpolar molecules that are insoluble in water but soluble in benzene, chloroform, hexane, methanol, and diethyl ether. To separate the components dissolved in the mixture, solvent extraction was used. Separation of particular compounds is achievable due to the solvent's ability to dissolve lipids. Methanol has a boiling point of 65 ℃. As a result, the solvent will evaporate at this temperature but the targeted

compounds will not. Due to this condition, lipids are readily separated. To assess the presence of free fatty acids in the extracted lipid, it was analysed using a gas chromatograph mass spectrophotometer (GC-MS). The table discusses the free fatty acids discovered during lipid analysis. These found free fatty acids indicated that food waste might be used to produce biodiesel.

4.4.1 Effect of Reaction Time

Table 4.1 Lipid yield by reaction time.

Time (min)	**Lipid Yield (%)**
10	14.5
15	17.3
20	18.5
25	20.0
30	17.0

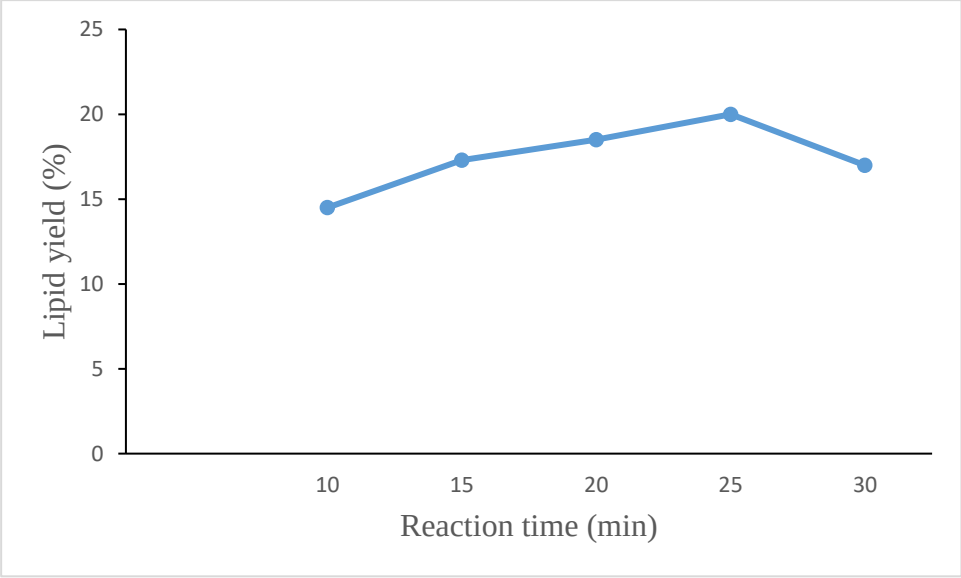

Figure 4.4 Lipid yield versus reaction time.

Figure 4.4 showed the lipid yield versus reaction time. This condition was performed in the fixed condition at 200 g of sample, 300 ml of solvent and 60 ℃. The effect of reaction time was observed using varying time parameter at 10-30 min. It was observed that the percentage of lipid yield increase at 10-25 min reaction time and decrease when reaction time exceed 25 min. In this study, the optimum reaction time for the lipid extraction is at 25 min with the highest lipid yield which is 20 %. It can be concluded that the reaction time is not directly proportional to the amount of lipid produced, but there is an optimum point in this reaction.

The temperature used is 60 ℃ which is relies on solvent boiling point for lipid extraction efficiency. This is because of the appropriate miscibility of methanol with lipid, it is preferable to perform the reaction close to the boiling point of methanol solvent (Barik *et al*., 2018).

4.4.2 Effect of Solvent Amount

Table 4.4 Lipid yield by effect of solvent amount.

Amount of Solvent	Lipid Yield (%)
300	37.0
350	28.5
400	26.0
450	77.0
500	95.0

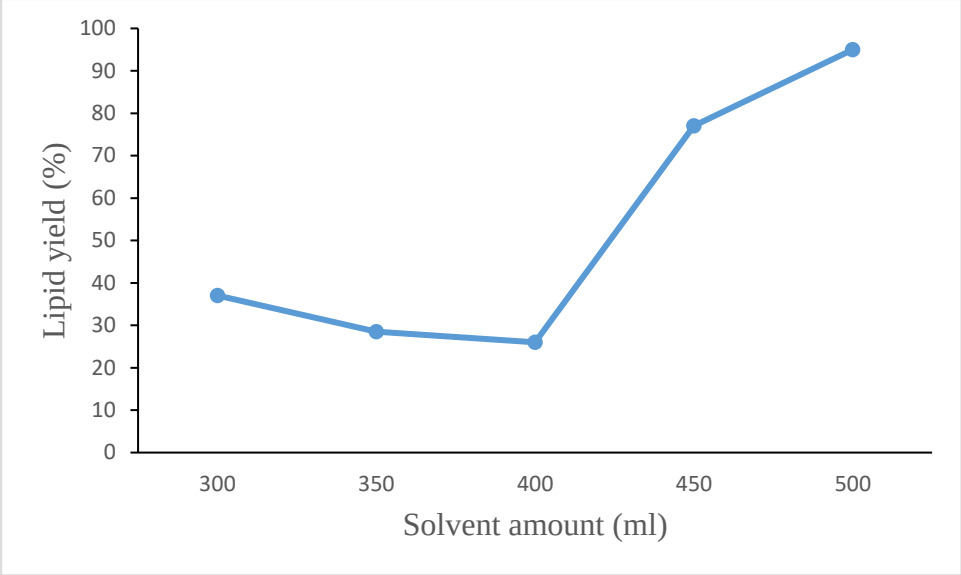

Figure 4.5 Lipid yield versus solvent amount.

Figure 4.5 showed the lipid yield versus solvent amount. The reaction was performed in the fixed condition at 200 g of sample, 60 ℃, and 10 min of reaction time with varying the solvent amount at 300-500 ml. It was observed that the percentage of lipid yield decrease when solvent volume in range of 300-400 ml and start to increase when solvent volume in the range of 450-500 ml. It was also observed that the highest lipid yield is at 95 %. Based on the results presented here, the optimal solvent volume for lipid extraction from food waste microalgae is 500 ml.

4.5 Free Fatty Acid (FFA) analysis

Fatty acids are carboxylic acids with a long hydrocarbon chain on one end and a carboxylic acid group on the other (usually between 4 and 22 carbons in length). In its finest form, a fatty acid has a completely hydrogen-saturated hydrocarbon chain (i.e., no double bonds). Mono-unsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) are fatty acids that have one or more double bonds in their hydrocarbon chain (PUFAs). Microalgae species utilised as feedstock for biodiesel production are selected suitably because saturated fatty acids create superior biodiesel. The most abundant saturated fatty acid in microalgae is palmitic acid (16:0), followed by stearic acid (18:0),

while the most abundant mono-unsaturated fatty acid is oleic acid (C18:0) (Dahman *et al*., 2019).

4.5.1 Analysis of free fatty acid (FFA) content at condition 25 min reaction time

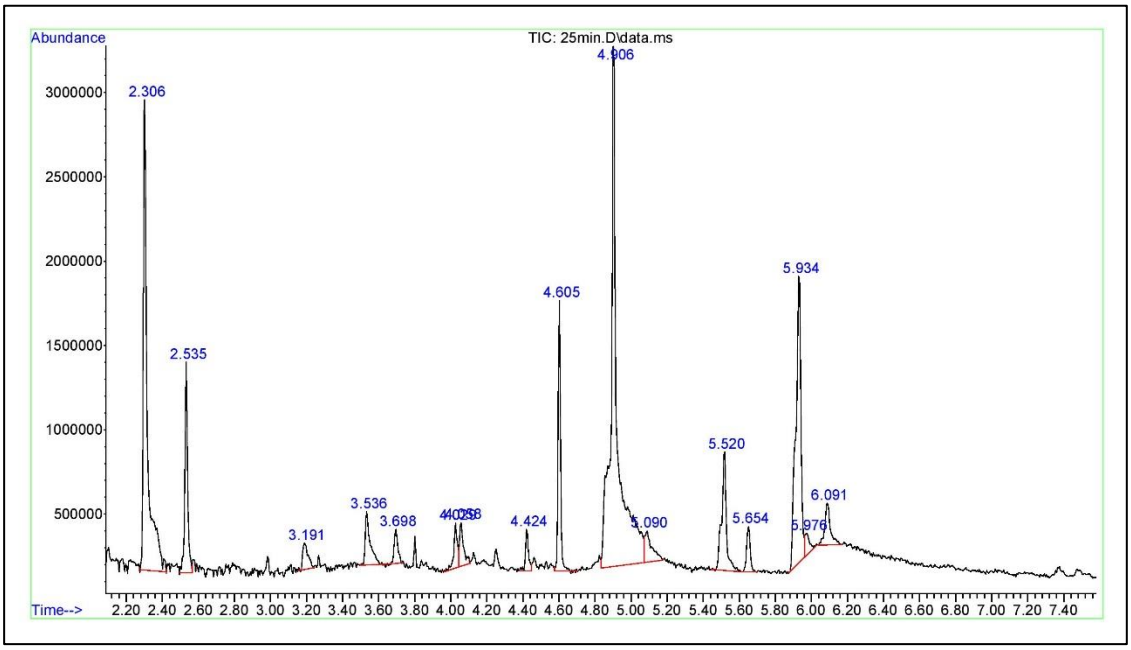

Figure 4.6 GC chromatogram at condition 25 min reaction time.

Table 4.3 The composition of free fatty acids at 25 min reaction time.

Retention Time	Identified Compound	Composition (%)
3.19	Lauric acid (C12:0)	1.70
5.09	Palmitic acid (C16:0)	2.13
5.98	Oleic acid (C18:0)	1.74

Table 4.3 showed the composition of free fatty acids (FFA) identified in lipid molecule. The parameter for this study at 200 g of sample, 300 ml of solvent, 25 min of reaction time and 60 ℃. It was observed that the total composition of fatty acids is 5.57 % with the highest composition of palmitic acid which is 2.13 %. Fatty acids identified may be both saturated and unsaturated. Saturated fatty acids identified include lauric acid (C12:0), and palmitic acid (C16:0). Unsaturated fatty acids have one carbon-carbon

double bond which can be present in different positions. The most common unsaturated fatty acids contain chain length of 17-22 and a double bond with cis configuration (Barik *et al.*, 2018). Unsaturated fatty acid identified in this study is oleic acid (C18:0). It was found that saturated fatty acids which is palmitic acid (C16:0) dominate in this analysis of lipid.

4.5.2 Analysis of free fatty acid (FFA) content at condition 500 ml of solvent amount

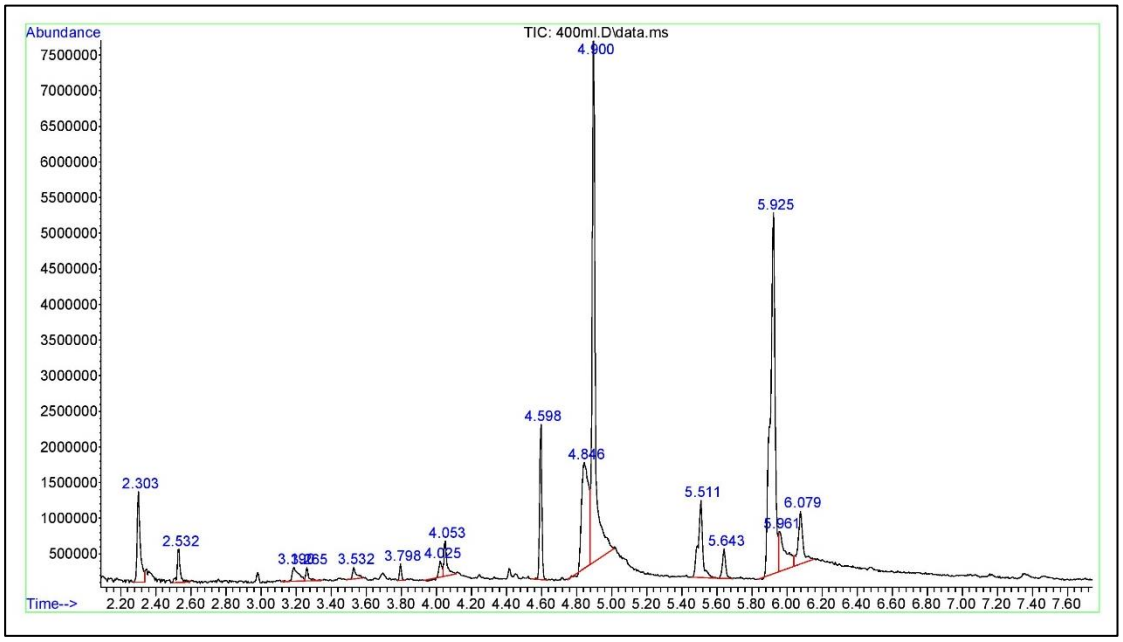

Figure 4.7 GC chromatogram at condition 500 ml of solvent amount.

Table 4.4 The composition of free fatty acids at condition 500 ml of solvent.

Retention Time	Identified Compound	Composition (%)
3.19	Lauric acid (C12:0)	1.55
3.53	Caprylic acid (C8:0)	1.10
4.03	Myristic acid (C14:0)	0.31
4.84	Palmitic acid (C16:0)	11.80
5.02	Palmitic acid (C16:0)	3.41
5.95	Oleic acid (C18:0)	2.65
6.01	Oleic acid (C18:0)	0.69

Table 4.4 showed the composition of free fatty acids (FFA) identified in lipid molecule. This study was performed in the condition 200 g of sample, 500 ml of solvent, 10 min of reaction time, and 60 ℃. It was observed that the total composition of FFA is 21.49 %. The FFA identified are lauric acid (C:12), caprylic acid (C:8), mystric acid (C:14), palmitic acid (C:16), and oleic acid (C:8).

The carbon chain length of fatty acids in the range of C8-C18 with minor quantity of C14. It was also observed that carbon chain length mostly within C16-C18 which are recommended for biodiesel production (Qiu *et al*., 2017). The dominant fatty acid was Palmitic acid (C16:0) in both condition that have been showed in Table 4.3 and Table 4.4. In that context, Palmitic acid (C16:0) also one of dominant fatty acids in freshwater microalgae that was cultivated for 7 days (Neofotis *et al*., 2016).

CHAPTER 5

CONCLUSION

5.1 Introduction

One of the most successful techniques for addressing the fossil fuel problem, environmental pollution, and energy demand is to replace non-renewable resources with renewable ones. Numerous research has been done to discover the best cost-effective alternative technique of recycling renewable resources, most notably waste products used in the production of biodiesel. Waste products such as sewage sludge, industrial trash, and used cooking oil are all available raw materials that may be used to make biodiesel, therefore minimizing waste generation, management, and disposal. This study has taken an approach to biodiesel production by using food waste. The optimum condition of microwave assisted extraction (MAE) method on lipid extraction of food waste microalgae is at 60 ℃, 500 ml of solvent, 200 g of sample and 10 min of reaction time resulting 95 % lipid yield.

A lipid study was done using GC-MS to detect the fatty acids. The free fatty acid (FFA) composition of food waste microalgae lipid molecules is C8-C18 carbon chain length with a minor amount of C8. It may be concluded that food waste microalgae have the ability to produce FFA that are important in the production of high-quality biodiesel. Microalgae have the potential to be a substantial and long-term renewable energy source that can fulfil global demand. Despite their many advantages, microalgae biofuels have certain disadvantages, such as low biomass yield and a small cell size that makes harvesting challenging. The development of advanced photo bioreactors and low-cost biomass collecting, drying, and oil extraction processes may be able to overcome these limits.

Furthermore, manipulating the metabolic pathways of microalgae through genetic engineering is an efficient strategy for enhancing biomass and biofuel output. The use of genetic engineering technologies in the production of high-value, low-cost goods is also crucial. In the development of microalgae biomass and biofuels, biotic interaction with bacterial biofilms is also crucial. However, most of this technology is still in development and has yet to be commercialised. As a consequence, greater research into innovative upstream and downstream technologies will help commercial microalgae biofuel production.

5.2 Recommendation

According to the findings of the research, microalgae biomass as pyrolysis feedstock have a number of long-standing difficulties. Despite the fact that microalgae is a third-generation biofuel feedstock that does not have the drawbacks that first- and second-generation biofuels have, its growth and harvesting still offer significant obstacles in terms of large-scale farming and cost. While open and closed system cultivation studies are significant with current global productions, the cost of production, particularly technological drying and pre-treatment, remains prohibitively expensive for industrialised. As a consequence, wet biomass microalgae must be dewatered before the pyrolysis process, which will raise the production cost. Microwave-assisted pyrolysis, which is capable of handling moderately wet samples to produce pyrolytic products, may be a solution to reduce the cost of pre-treatment in this case.

However, using just one pre-treatment method may not be enough to achieve the highest lipid extraction yield from lysed biomass, so it may be beneficial to use multiple pre-treatment methods on both a lab and large-scale basis. If ultra-sonication and microwave irradiation are used combined for cell disruption under wet condition, for example, four separate physical processes will act together to readily rupture the cells and release the lipids to the external environment. When ultrasonic waves are transmitted, microbubbles form during the rarefaction phase of the sound wave and collapse during the compression phase, resulting in cavitation. Shock waves in the form of mechanical energy are released by disintegrated microbubbles, causing irreversible shearing in the cell walls of oleaginous microorganisms. Following the ultra-sonication step, microwave

treatment causes polar compounds in cellular compartments to align in the direction of the applied electric field, which is followed by high-speed rotation as the microwave field changes. The time of the procedure as well as the amount of solvent used might be reduced with this approach combination.

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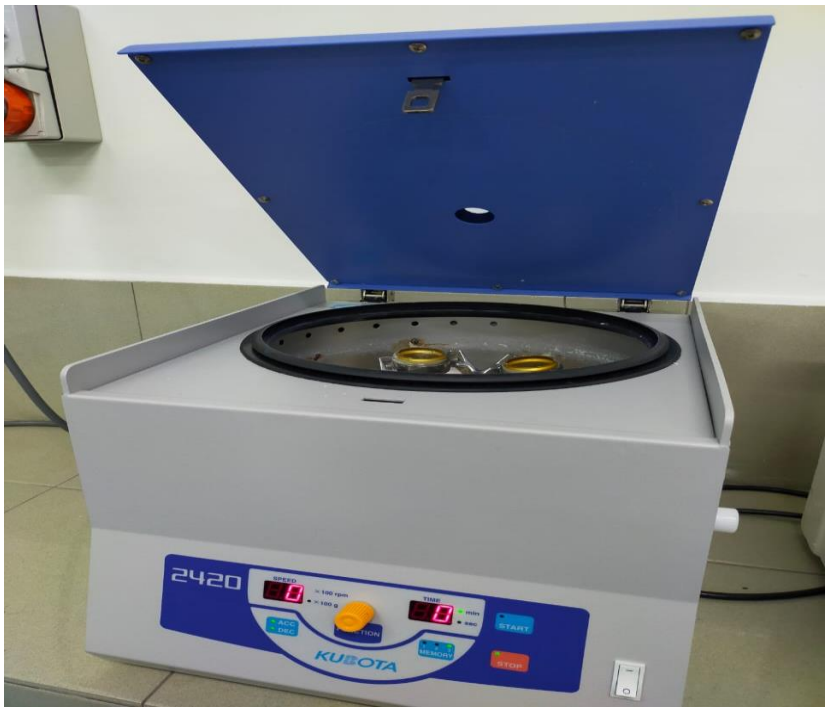
APPENDICES

APPENDIX A: CENTRIFUGE PROCESS



APPENDIX B: MICROWAVE EXTRACTOR