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Ramification of alcohols towards microalgae fatty acid esters recovery via direct transesterification

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ABSTRACT

Alcohols are commonly utilised for methyl or ethyl esters production in the transesterification of biodiesel production; a non-petroleum-based energy sources which is mostly cited as mono-alkyl esters. The substantial needs of high reactant concentration and catalyst have diverted studies towards direct transesterification (DT). Traditional physicochemical disruption towards microalgae cells which aims for free fatty acids collection commonly goes all along with the DT which reducing a lot of cost and chemical dependency. The aimed fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs) were transesterified using freshly harvested Chlorella sp. 15g/L HCDs of wet microalgae and alcohol with ratio of 1:3 microalgae oil to alcohol were used in utilising the DT. This method could provide higher reaction rate as well as a shorter reaction time and promoting a higher purity of biodiesel. The objective of this study is to observe the effect of ethanol and methanol as acylation agent in producing biodiesel via direct transesterification of *Chlorella* sp. The Fourier-Transform Infrared Spectroscopy (FTIR) and Gas Chromatography Mass Spectrometry (GC/MS) were used to determine the purity and the characteristics of the alcohol affecting the DT. The biodiesel from methanol solvent provides higher purity product since it was more soluble and less dense compared to the ethanol, which enhanced the reactivity of DT. The final mass of FAME was lower compared to FAEE; 1.528 gram and 2.691 gram, respectively. This is due to the simple structure of methanol, which enhanced the bond breaking as well as vaporisation at a lower temperature. Final FAME product was characterised as butenoic acid, 3-methyl-, methyl ester with purity of 37.84 %, and FAEE product was a 9-octadecenoic acid (Z)-, methyl ester with purity of 15.75 %. In conclusion, direct transesterification using methanol was more effective than ethanol due to its molar ratio.

1. INTRODUCTION

Energy plays a crucial role in driving a nation's economic and social progress. Looking ahead to 2050, it is projected that over 90% of the energy will be derived from low-carbon sources, with fossil fuels

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contributing less than 10% (Summary of the National Energy Transition Roadmap Phase 1 Solidifying Energy Aspirations, 2023). Renewable energy sources will take centre stage as the primary fuel for the economy, while fossil fuels will still be necessary for sectors that are challenging to decarbonise (Summary of the National Energy Transition Roadmap Phase 1 Solidifying Energy Aspirations, 2023). However, the rapid consumption of fossil fuels is alarming, and their reserves are depleting. As the demand for energy and global fuel prices continue to rise, alternative sustainable energy sources like biodiesel are gaining popularity. This is primarily due to the growing concern about global warming and the detrimental effects of immense carbon dioxide (CO_2) and greenhouse gas emissions (GHGs) resulting from various human activities. These emissions contribute to environmental pollution and the degradation of natural ecosystem, exacerbating global warming (Ben Bacha et al., 2022 ; Banković–Ilić et al., 2017).

The impact of trace gases on climate change is significant and cannot be ignored. It is crucial to reduce these emissions, and an effective way to accomplish this is by implementing strategies that prioritise renewable energy sources. There is an urgent need to find a sustainable, affordable, and eco-friendly energy source. Biodiesel has undergone extensive research as a viable alternative with reduced carbon emissions. Using biodiesel instead of petroleum fuels, especially in transportation, reduces CO_2 and other greenhouse gas emission (Osman et al., 2021). The depletion of the world's oil reserves is directly caused by the ongoing use and extraction of biodiesel, which has become a challenge for conventional sources of edible oil due to the increase in population and the expansion of the biodiesel industry. Given the perpetual rise in global energy requirements, the most effective approach to address this escalating demand is to seek out substitute sources of fuel.

Numerous studies have focused on the use of solvent systems in biodiesel production. As vegetable oils are frequently utilised to produce biodiesel; however, their high price has prompted researchers to explore alternatives that are more affordable (Taher et al., 2011). Waste oils, cooking oils, and animal fats have been suggested as alternative feedstocks. However, animal fats have the disadvantage of having high melting points, which may necessitate the use of organic solvents. Yet, using organic solvents requires energy and solvent recovery equipment to be involved during the separation process. Also, these feedstocks are ineffective and harmful to the environment (Srivastava & Prasad, 2000). On the other hand, microalgae have emerged as a promising alternative for biodiesel production. These organisms have the ability to thrive via photosynthesis and accumulate large amounts of lipids.

Microalgae have drawn the attention of food industry researchers as a feasible and promising alternative for producing fuel and food. A sustainable and efficient source of edible oil that contributes to the preservation of agricultural resources is microalgae. Microalgae aids in the preservation of valuable agricultural resources (Xue et al., 2020). Algae are aquatic organisms that utilise photosynthesis to grow and thrive. Algae have a high level of adaptability and can thrive in diverse environments with differing temperatures, salinity levels, and pH values. They can be found in a range of water bodies such as rivers, lakes, oceans, and even wastewater. They are able to grow individually or in symbiosis with other organisms (Barsanti et al., 2008). The oil yield from microalgae can reach up to 6,000–15,000 gallons of oil per acre, surpassing the production of vegetable oil by a large significant amount due to their exceptional lipid productivity (Sajjadi et al., 2018). Also, it can double its biomass within 24 hours which can produce high oil content, exceeding 80% by weight of dry biomass (Chisti, 2007).

Fresh-water algae (*Chlorella* sp.) are utilised as a raw material in the production of biodiesel since it can provide high lipid yields that can attract a lot of attention as a clean and sustainable source of biofuel. As compared to alternative methods, the use of bio-flocculation and wastewater-cultivated microalgae has indicated a substantial advancement in biomass recovery technologies. Biomass cultivation, harvesting, oil extraction, and transesterification are the general steps to produce biodiesel from algae (Inayat et al., 2019). Microalgae only produce small amounts of lipids under optimal growth conditions, but they store lipids as

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a source of carbon and energy when the organism is stressed by its surroundings. As a result, it improves microalgae biomass, lipid productivity, and production efficiency while keeping production costs to a minimum (Ratnapuram et al., 2018).

Direct transesterification (in-situ) of oil and alcohol involved in this study is utilised to exchange the R-group from an alcohol with the R'-group from an ester through the addition of an acid or base catalyst to the reaction mixture. Sodium hydroxide (NaOH) is acting as base catalyst to helps achieved in high yield of biodiesel. The transesterification from different types of alcohols causes significant reaction time, production costs and conversion procedures. The most well-known alcohols that are used in the transesterification process are methanol and ethanol. Short chemical chain structure of methanol and ethanol facilitate faster, and more efficient transesterification reactions compared to longer-chain alcohols (Farouk et al., 2024). Moreover, direct transesterification reactions require solvents such as alcohol to break down the cell wall and directly react with the lipid in producing biodiesel (Pandey et al., 2024). Hence the extraction process would be eliminated which can reduce the time consumption and the cost of operation. To make the production of microalgae more economical, and efficient combination of waste exhaust carbon dioxide (CO_2) and wastewater streams was suggested. CO_2 is utilised due to its beneficial characteristics, which encompass its non-flammable, non-toxic, and non-corrosive nature in the presence of water (Yerena-Prieto et al., 2022). Moreover, renewable resources offer a plentiful supply of CO_2, ensuring high purity and less costs. As a result, it enhances the solubility of the oil-alcohol mixture, reducing transportation obstacles and improving reaction rates (Tobar & Núñez, 2018).

2. EXPERIMENTAL

2.1 Chemicals and materials

Chlorella sp. powder (Algae Living Sdn Bhd), calcium chloride (CaCl) (Sigma-Aldrich), sodium hydroxide solution (NaOH) (Sigma-Aldrich), sodium chloride (NaCl) (Sigma-Aldrich), methanol (CH_3OH) (Sigma-Aldrich), ethanol (CH_3CH_2OH) (Sigma-Aldrich) and n-Hexane (Sigma-Aldrich).

2.2 Microalgae cultivation

Chlorella sp. powder was mixed with tap water with the ratio of 1:1 (w/v) (g/L). The medium was fed with NaCl powder with the ratio of 1:0.5 (w/w) of NaCl to *Chlorella* sp. powder on the first day and fifth day during the cultivation of microalgae (Insan et al., 2018). According to Sikorski, (2021), NaCl can hinder the growth of algae by controlling the osmosis stress; therefore, would help to generate cells in order to produce more lipids. It necessitates engaging in photosynthesis while it is being subjected to the sunlight and carbon dioxide (CO_2). The tank, positioned in proximity to windows, was exposed to sunlight. Aeration pump with a low-pressure condition were introduced into the medium to provide CO_2 from the air and experimental conditions were maintained at a consistent room temperature of 25 °C to establish an environment conducive to algae cultivation. Sunlight can enhance the production of fatty acids in the microalgae and their suitability as biodiesel sources.

At first microalgae was cultivated to observe its growth rate. 1L of cultivation medium were harvested every day to observe their wet biomass. The growth rate observation method was done until the growth curve showing the death phase of these microalgae. The growth curve has shown the highest peak of microalgae growth phase which indicates the highest amount of wet biomass. Next, recultivation was done for several days which is until its highest wet biomass weight that base on the growth curve data.

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2.3 Microalgae harvesting and cell breaks

A volume of 1L of the medium was centrifuged at 4,500 rpm, 10 °C, for 10 min to separate the microalgae from the culture medium (Tan et al., 2018). The separation process was done to collect concentrated samples of wet algae. The weight of wet algae was recorded in gram (g). A high concentration dilute solution (HCDs) approach was employed, wherein tap water was added to the collected wet algae sample until the resulting mixture reached a mass concentration of approximately 15 g/L. The weight of (HCDs) wet microalgae was recorded and was refrigerated at a temperature range of 1-4 °C for a period of 24 hours prior to undergoing direct transesterification process.

2.4 Direct transesterification (DT)

The direct/in-situ transesterification (DT) was a method of producing biodiesel by integrating lipid extraction and transesterification within a single step (Eq. (1)). This method helps to reduce time, costs and environmental effects due to the minimal use of a few solvents and reagents in this process (Torres et al., 2017). It has many benefits, rendering it a promising technique for having a sustainable biodiesel production.

$$\text{Microalgae FFA} + \text{Alcohol} \rightarrow \text{Glycerides} + \text{Esters} \quad (1)$$

This study utilised two samples, each employing a different type of alcohol (methanol and ethanol) as solvent, to analyse the different alcohols' impact on the transesterification product as detailed in this study. Each sample of 15g/L HCDs wet microalgae was mixed with alcohol (methanol or ethanol) by ratio of 1:3 microalgae oil to alcohol. NaOH catalyst then were added into the mixture with 1-2 wt% (ml/ml) of 0.1M NaOH. Next, the cells were obstructed using sonification for 20 minutes in ultrasonic bath (Grant XUBA 1). This technique was highly effective for disrupting the cell walls of *Chlorella* sp. and extracting lipids. Also, it helped to enhance the efficiency of the absorption of the solvent into the cells (Gerde et al., 2012).After being sonicated the sample was heated at temperature of 55 °C, with a rotation speed of 150 rpm, for a duration of 3 hours on a hot plate.

2.5 Purification

The direct transesterification mixtures were then centrifuged at 4,500 rpm at room temperature for 10 min to eliminate impurities and gather the products containing both types of alcohols. The separation results in two layers, aiding in the collection of the denser component by allowing it to settle at the bottom of the tube.

An amount of 50 ml of Hexane (n-hexane) was introduced into the collected samples to extract water from the mixture, enhancing the separation of the final products (FAME and FAEE) from glycerol and by-products (Fig. 1). The sample was allowed to sit for 15 min before repeating the same procedure in order to enhance the effectiveness of the separation. The uppermost layer (non-polar layer), consisting of a hexane mixture, was collected in order to continue with the subsequent washing process. The washing process was done by introducing 50 ml of distilled water to the hexane mixture (previous non-polar layer) and collect the upper layer which contained any possible types of biodiesels.

In the production of biodiesel, it is important to perform repeated washings to remove impurities like soap and water that were produced as by products. Thus, the washing procedure was carried out three times in 15 min for each batch to ensure the attainment of a high level of purity for FAME and FAEE, while simultaneously eliminating any remaining residues within the mixture. A washing method is used on the collected hexane (top layer) to eliminate any potential impurities in the biodiesel solution such as glycerol and soap (Fig. 1).

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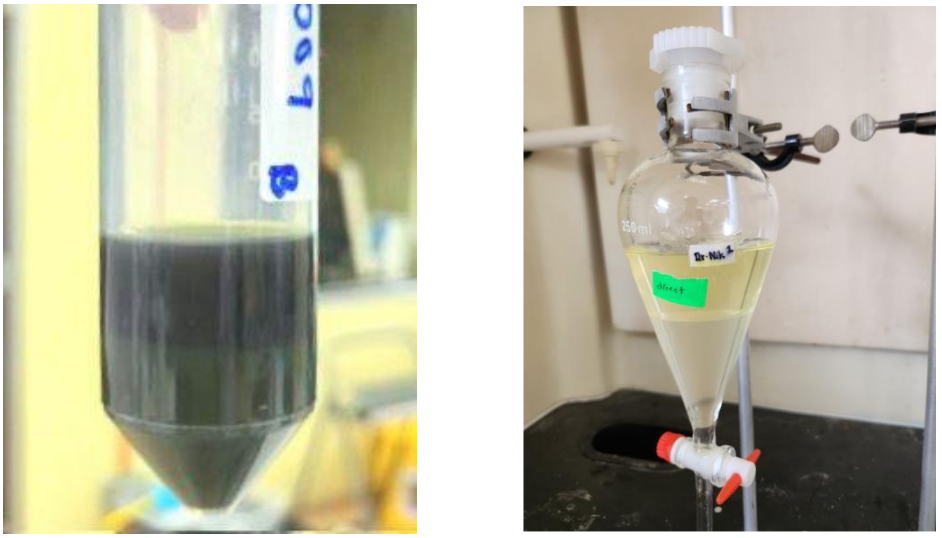

Fig. 1. (left) Separation of biodiesel mixture from the microalgae; (right) washing method to remove any residual inside the mixture

Source: Authors' own images

2.6 Anhydrous filtration using calcium chloride

The biodiesel mixture was collected for a filtration process. Anhydrous filtrations were utilised in order to absorb moisture, which helped to remove the remaining water content from the biodiesel. Method was done according to (Sharma et al., 2019). To prepare the FAME and FAEE product, the collected hexane was mixed with 5.0 g of anhydrous calcium chloride (CaCl). After letting the mixture sat for approximately 10 minutes, it was filtered through filter paper.

Next, both biodiesel mixtures, the FAME and FAEE were heated at 40 °C to eliminate any leftover moisture and excess hexane using a hot plate in a fume hood until the mass was constant. This procedure was carried out in order to obtain only the FAME and FAEE yields. Subsequently, the FAME and FAEE solution, with a volume around 10 ml (collected after heating process), was transferred to a universal bottle and labelled accordingly (Ichihara & Fukubayashi, 2010)

2.7 Fourier Transform Infrared Spectroscopy (FTIR)

The concentration of biodiesel and oil was predicted by developing a calibration model FTIR during the biodiesel analysis (Mahamuni & Adewuyi, 2010). It was also used to detect functional groups that represents alcohol. The sample was extracted from the scintillation vial by using a pipette and was transferred onto the IR crystal of the spectrometer for about 1 or 2 drops. Perkin Elmer's Fourier Transform Infrared Spectroscopy (FTIR) was used for the analysis. Approximately 5 min of total time is needed for the spectral collection. The range of the spectra was obtained over 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1}. The average of 20 scans for each spectrum represented and was corrected by subtracting the background.

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2.8 Gas Chromatography Mass Spectrometry (GC/MS)

GC/MS was a universally employed method for analysing the levels of fatty acids and alkyl esters in both the source (oil or triglycerides) and the product (biodiesel) of the in-situ transesterification process. Its primary purpose was to assess the conversion of biodiesel by accurately determining the compounds generated throughout the process (Ponnusamy et al., 2020). Agilent 7890A GC/MS was used for the GC/MS analysis. The gas flowrate was set at 1 ml/min. Injector temperature was set at 250 °C. Oven temperature was hold at 60 °C for 3 minutes. The temperature was increase to 120 °C at 10 °C/min. It is then rose up to 250 °C at 5 °C/min and hold for 10 minutes. The total operating time is around 45 minutes. Mass Spectrometer scan range was from 50m/z to 500m/z.

3. RESULTS AND DISCUSSION

3.1 Microalgae cultivation, harvesting and growth evaluation

This microalgae exhibits diverse applications in biotechnology, including bioremediation and biofuel production. Its photosynthetic nature enables efficient conversion of light energy into biomass. Through meticulous cultivation and precise control of growing conditions, its production and utilisation across various industries have been optimised (Farhangi et al., 2023) and reported scientifically.

Microalgal growth and biomass production was affected by nutrient availability, temperature, and light; where the latter being the most crucial ones, which enhanced the productivity of both the biomass and the desired bioproducts. The conditions have induced metabolic changes during the culture phases. This means that ensuring enough and right concentration of suitable nutrients or growth medium during this phase is of utmost importance for successful microalgal biomass production (Figueroa-Torres et al., 2021). *Chlorella* sp. cultures have been cultivated for the total of 15 L of tap water in low-pressure aeration tank function for 11 days and the highest growth of algae has been observed. Based on Fig. 2, the cultivation of *Chlorella* sp. using tap water was plotted. Through observation, the mass of wet biomass started to increase from Day 1 until Day 10, and then drop at Day 11. There was no reduction in growth was spotted before Day 11 due to addition of 7.5 g NaCl on Day 5. NaCl provides ions of Na^+ and Cl^-, which play roles in cellular ion balance and osmoregulation in the culture medium. This condition has helped the microalgae to increase lipid content (Sikorski, 2021). The increases weight of biomass indicates that the medium culture contains enough nutrients for the algae growth. Day 10 has the heaviest wet algae weight (7.954 ± 0.08 g). Higher weight of wet algae would produce higher lipid content. Thus, the sample of wet algae on Day 10 was used for the D/IS-T.

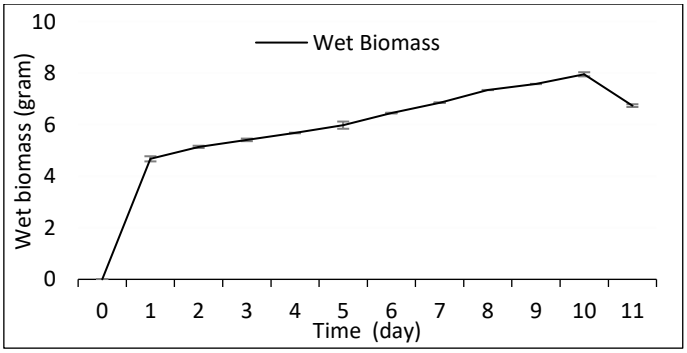

Fig. 2. Growth curve and cultivation of the *Chlorella* sp.

Source: Authors' own data

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3.2 Identification of the FAME and FAEE

FTIR analysis was conducted to identify the contents for each sample that occurs in FAME and FAEE, for an accurate characterisation of their components (Fig. 3 and Fig. 4). The main functional groups that were expected to appear were the C-H group, O-H alcohols and acids group, and C-O group. These groups were compatible to the structures of $CH_3(CH_2)nCOOCH_3$ (FAME) and $CH_3(CH_2)nCOOC_2H_5$ (FAEE) (Zulqarnain et al., 2023). FTIR employed to identify the presence on specific chemical groups in the compounds, to determine their chemical structure, to monitor chemical reactions, and to assess the purity of substances (Li et al., 2025; Gong et al., 2024)]. Therefore, this method was significant to be used.

The FTIR graph for FAEE (Fig. 4) shows the same patterns as in Fig. 3 (FAME). The highest peak for C-H group was seen at 2956.22, followed by the small peaks of 2922.09 and 2873.21, which in the range of ester functional group, followed by hydroxyl group and carbon group (Mike Braley, 2015). Therefore, the production of FAME could be verified as production of biodiesel as both FAME and FAEE have similarity on C-H bond, C-O bond and -OH bond.

To determine whether the sample contains fatty acid methyl ester (FAMEs), GC/MS analysis had been done to double confirm the presence of FAME and its functional group from FTIR analysis. Fig. 5 shows that 2-butenoic acid, 3-methyl-, methyl ester was recorded as the possible component that presence in the produced FAME. It contained fatty acids with the same formula and methyl ester group. Butenoic acid has double bond between two adjacent carbon atoms in the molecular structure ($CH_3CH=CHCOOH$). It was also categorised as carboxylic acids group and have a chemical formula of (-COOH) within the molecule, which have similar properties with fatty acids formula long-chain hydrocarbons with a carboxyl group (-COOH) as shown in Fig. 6.

Next, FTIR data for FAEE analysis from Fig. 4 also need to be confirmed. Hence GC/MS analysis had been done to double confirm the presence of its ester functional group. Fig. 6 shows that the direct transesterification by using ethanol producing 9-octadecanoic acid (Z)-, methyl ester as its ester product. Ethanol with chemical formula of CH_3CH_2OH may be breaking down to CH_3OH during the transesterification process (due to heating) or during the sonication process before it is transesterified with fatty acid. Octadecanoic acid (stearic acid) chemical formula was $CH_3(CH_2)_{16}COOH$ which indicates a fatty acid with 18 Carbon chain. Hence, direct transesterification of microalgae using ethanol proof to produce a high-quality biodiesel.

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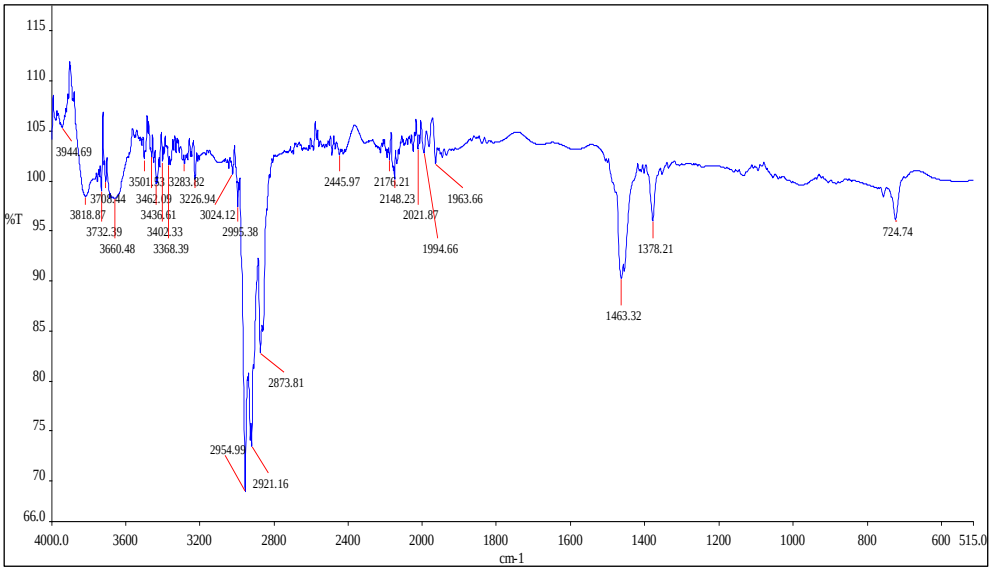

Fig. 3. FTIR analysis on FAME

Source: Authors' own data

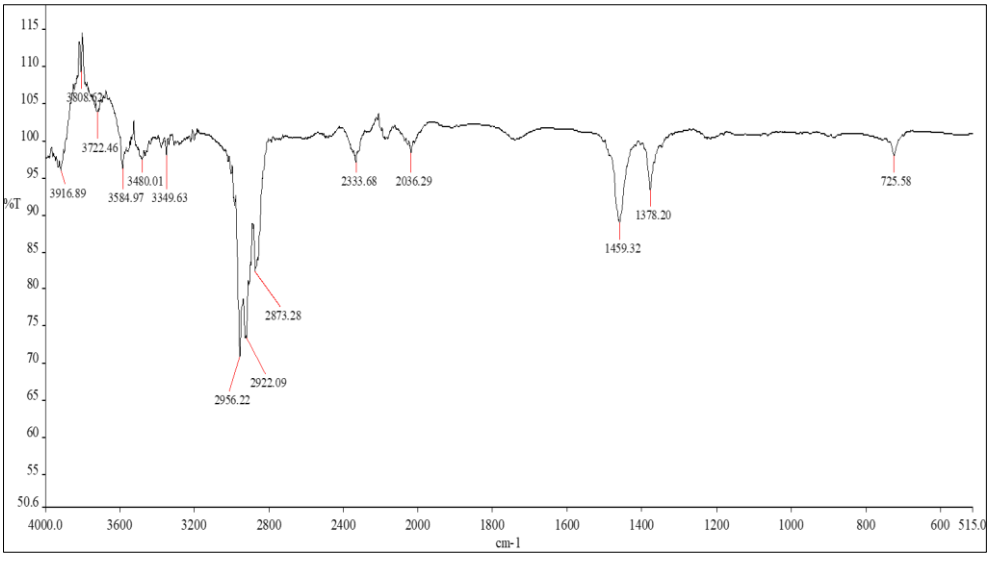

Fig. 4. FTIR analysis on FAEE

Source: Authors' own data

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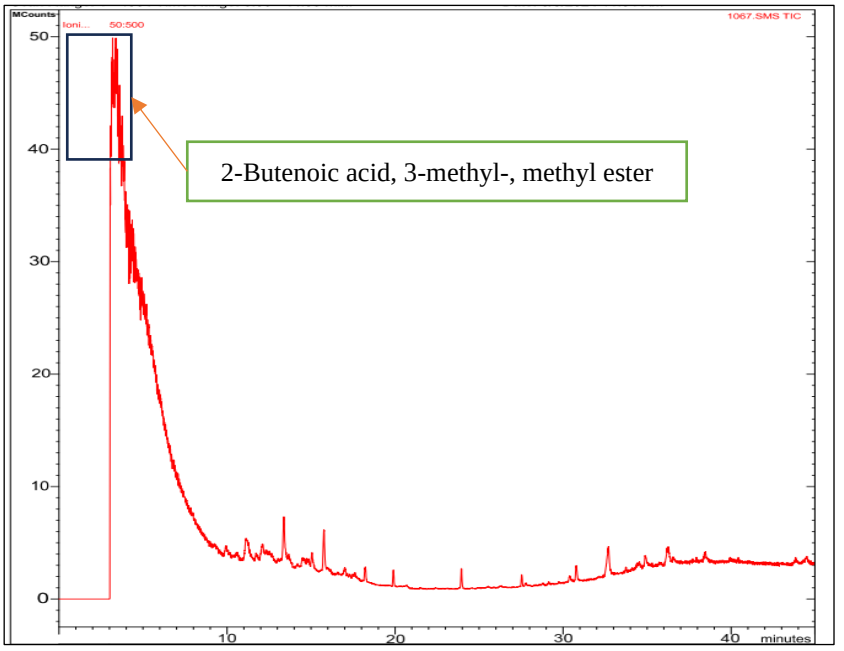

Fig. 5. GC/MS analysis for 2-butenoic acid, 3-methyl-, methyl ester

Source: Authors' own data

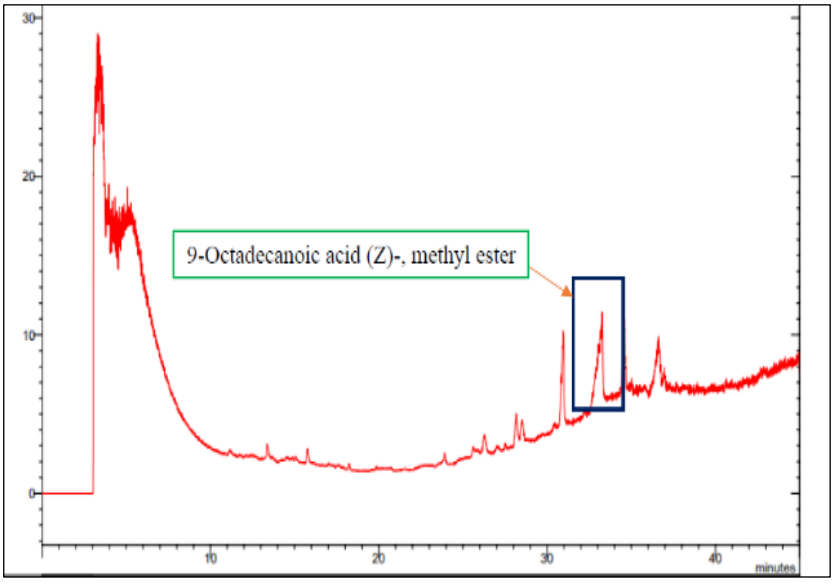

Fig. 6. GC/MC analysis on FAEE (9-Octadecanoic acid (Z)-, methyl ester)

Source: Authors' own data

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From the tabulated data in Table 1, the purity of each molecule for FAME and FAEE were recorded to be 37.84% and 15.75%, respectively. According to Helwani et al. (2009), high purity leads to high reactivity. High purity will reduce the impurities in the production, which also helps induce side reactions. The increase of reactivity will shorten the time taken of the reaction, enhance the production and produce high yields of the product. Thus, FAME was more substantial compared to FAEE. Apart from this, previous research has shown that utilizing methanol as the raw material leads to increased biodiesel yields, purity and higher lipid content, ultimately resulting in a high purity biodiesel product (Salaheldeen et al., 2021).

Table 1. GC/MS of FAME and FAEE analysis

	FAME	**FAEE**
Chemical compound	Butenoic acid, 3-methyl-, methyl ester	9-Octadecenoic acid (Z)-, methyl ester
Molecular Formula	$C_6H_{10}O_2$	$C_{19}H_{36}O_2$
Molecular Weight (g/mol)	114.14	296.4879
Retention Time (min)	3.9786	34.5337
Purity (%)	37.84 %	15.75 %
Base Peak	76 m/z (79)	201 m/z (95)
Min. Abund	0.10%	0.61%
Area (%)	0.0655%	0.0421%
Scan	283	3304
Peak Tailing	02.3	3.0
S/N (total)	7783	3034

Source: Authors' own data

The data presented in Table 2 indicates the final mass of FAME and FAEE after the production process. The final mass of FAME was recorded to be 1.528 gram and FAEE was 2.691 gram. Research by Loh et al. (2021) reported FAMEs recovery was 0.0501 gram and 0.0683 gram from freshly harvested *Chlorella* vulgaris using a conventional oil-extraction-transesterification (OET) and direct transesterification (DT), respectively. Thus, FAME and FAEE (produced through DT) were superior by 4 to 5 times greater compared to the earlier reports. FAME, produced using methanol (CH_3OH) as solvent, has a simple structure and low boiling point compared to the FAEE that used ethanol (C_2H_5OH) as solvent. In contrast with the ethanol, methanol has weaker intermolecular forces; making it easier to evaporate and vaporised at lower temperature thus fasten the reaction rates. This, purity is not the only reason reactions or loss of bonds are affected.

Table 2. Product mass of FAME and FAEE

Item	**FAME (g)**	**FAEE (g)**
Mass of the bottle	17.737	15.745
Mass of the sample before heating + bottle	24.875	27.594
Mass of the sample after heating + bottle	19.265	18.436
Mass of the product after heating	1.528	2.691

Source: Authors' own data

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The effect of methanol and ethanol as solvents using the D/IS-T method can be discussed through characteristics of the produced fatty acid esters. There was slight difference between the characteristics of FAME and FAEE. The molar ratio of 3:1 was not suitable for producing FAEE due to limited carbon content in ethanol to transform into ethyl ester. As a result, fatty acids groups did not be able to react with the ethanol, resulting in lower purity of FAEE (Table 3). According to Pydimalla et al. (2023), the optimal range ratio of alcohol:oil for FAEE production is 3:1 or 4:1 with a consumption of higher energy and suitable condition to generate FAEE. This allows for efficient in-situ transesterification, minimizing byproduct formations, and maximizing FAEE yield and purity.

Table 3. Summary on the effect of methanol and ethanol in the biodiesel production

Characteristics	FAME	FAEE
Drying rate	The biodiesel product reach constant weight after 2 hours of heating process (faster)	The biodiesel product reach constant weight after 4 hours of heating process (slower)
Effectiveness	High	Low
Molar ratio effect	Effective	For 1:3 molar ratio of oil to alcohol, higher temperature was needed for ethanol solvent to produce FAEE
Chemical compound	Butenoic acid, 3- methyl-, methyl ester	9-Octadecenoic acid (Z)-, methyl ester
Purity	37.84 % (high)	15.75 % (low)
Colour	Pale yellow to green	Pale green
Environmental concern	Eco-friendly compound due to its higher carbon-neutral or carbon-negative characteristics	Considered relatively safe for the environment

Source: Authors' own data

Table 4 listed basic important characteristics recorded and gained during this research. The given chemical compound characteristics have been compared with the PubChem and was accepted to be significant.

Table 4. The FAME and FAEE

FAME	Characteristics	FAEE
Butenoic acid, 3-methyl-, methyl ester	**Chemical compound**	**9-octadecenoic acid (Z)-, methyl ester**
$C_6H_{10}O_2$	Molecular formula	$C_{19}H_{36}O_2$
114.14	Molecular weight (g/mol)	296.5
233 – 235	Boiling point (°C)	245 – 250
0.89 – 0.90	Density (g/mL) at 20°C	0.92 – 0.93
3.8 – 4.2	Viscosity (mm^2/s) at 40°C	4.2 – 4.5

Source: National Center for Biotechnology Information PubChem (2025)

4. CONCLUSION

The characterisation for both biodiesels has been observed. The final mass of FAME was lower compared to FAEE due to the simple structure of methanol, which enhanced the bond breaking as well as vaporisation at a lower temperature. Methanol was more effective in producing high purity of biodiesel than ethanol due to its molar ratio. The FAME produce by methanol was characterised as butenoic acid, 3-methyl-, methyl ester with purity of 37.84 %, and the FAEE produce by ethanol was a 9-octadecenoic acid (Z)-, methyl ester with purity of 15.75 %. The different type of biodiesel produce from this study can provide a significant information that can be applied in industry. However, it is still limited to lab scale production and need further investigation to scale up into industrial production.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no financial, professional, or personal interests that could be construed as influencing the content, results, or interpretations presented in this research.

AUTHORS' CONTRIBUTIONS

Muhammad Syafiq Abu Hassan: Conceptualisation, methodology, formal analysis, investigation and writing-original draft and editing; **Aina Irdina Mohd Yusmadi**: Conceptualisation, methodology, investigation, formal analysis, writing-original draft and editing; **Nik Raikhan Nik Him**: Conceptualisation, supervision, writing- review and editing, and validation.

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