

Energy efficient direct transesterification of *Nannochloropsis* sp. using hydrodynamic cavitation

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ABSTRACT

The increasingly limited supply of fossil fuels requires renewable fuel as an alternative source. *Nannochloropsis* sp. is a microalgae species containing a lipid content of between 12 and 53%, which can be converted to biofuel as an alternative source of fossil fuels through a transesterification process. Up to this date, the literature has reported no studies on biodiesel production from *Nannochloropsis* sp. via direct transesterification with catalyst using hydrodynamic cavitation. The direct transesterification process introduced 7.5 g of microalgae, 40 ml of methanol, 90 ml of hexane, and 0.0225 g of sodium hydroxide into the sample chamber. These mixtures were passed within the cavitation using a pressure driver and transformed into fatty acid methyl ester (FAME). The catalytic hydrodynamic cavitation method produces a higher extract yield than the stirring one. Regarding the FAME composition, the catalytic hydrodynamic cavitation method is dominated by saturated fatty acid (palmitic), while the stirring catalytic method is dominated by monounsaturated fatty acid (oleic). The hydrodynamic cavitation method provides a lower average degree of unsaturation and shorter chain length than the stirring catalytic method.

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1. INTRODUCTION

The rapid growth of the global population in recent years has led to an increase in fuel consumption [1], [2]. Every nation requires the energy to prosper economically [3]. The primary energy source is fossil fuels such as petroleum, coal, and natural gas. The increasing energy demand has resulted in a greater reliance on fossil fuels. Fossil fuels supply 80% of the world's energy needs. Fossil fuels are non-renewable energy sources because their natural sources are finite and are rapidly depleted due to uncontrolled consumption [4]–[6]. That's why searching for alternative sources will be crucial in the coming years [3]. Renewable fuels are an alternative to substitute limited primary fuel sources [7].

Many aspects of daily life depend on fuel, particularly the transport of people and goods. Mass transportation commonly uses diesel fuel, but a blend of biodiesel and diesel is becoming more prevalent. Biodiesel is a renewable fuel that can be produced from lipids found in microalgae. Microalga is an alternative to switch fossil fuel consumption [8], [9]. Microalgae are unicellular or multi-cellular microorganisms that live in restricted aquatic environments. Because they are major oxygen producers, they account for 40% of the oxygen supply in our ecosystem, and their ecological roles are highly significant [10].

Microalgae are very simple plants that do not have roots, stems, and leaves but have chlorophyll and can grow in seawater and fresh water. More than 40,000 species have been isolated and analyzed to date. Microalgae contain various compounds, mainly in the form of proteins, lipids, and carbohydrates [11]. Several microalgae species could be utilized as feedstock for biodiesel because they contain a significant amount of lipids [12], one of which is the *Nannochloropsis* sp., whose lipid content ranges from 12 to 53% of the microalgae's dry weight [13], [14]. Microalgae lipids contain triglycerides, which can be used in biodiesel production because the number of carbon atoms forms lipids with 14 to 20 carbon atoms [15].

In addition to being rich in bioactive compounds, microalgae offer several other benefits that make them highly versatile: i) they grow in water, which helps conserve land used for agriculture and alleviates land pressure, ii) microalgae grow and reproduce faster than many other plants and can thrive in more extreme conditions, iii) in agriculture, they can enhance soil fertility by promoting nutrient cycling, positively impacting plant growth, and iv) microalgae also help mitigate global warming by absorbing carbon dioxide during photosynthesis. Furthermore, beyond oil, the production of bio-oil from microalgae biomass waste adds value as an energy resource [16]–[18]. In general, microalgae hold significant promise for future growth and application.

Making biodiesel from microalgae involves several processes, namely cultivation, harvesting, lipid extraction, and trans-esterification reactions; among many processes, lipid extraction is the most energy-intensive step [19]. Therefore, this step makes biodiesel production from microalgae economically unviable. Various lipid extraction methods have been developed to reduce energy requirements: mechanical stirring, supercritical methanol, microwave heating, hydrodynamic cavitation, and ultrasonic cavitation. Among these methods, hydrodynamic cavitation (HC) is an effective method for the extraction process [20]. Hydrodynamic cavitation is one of the innovative technologies for biofuel production and wastewater processing [21], [22]. A further benefit of using the hydrodynamic cavitation approach is that it can effectively mix two immiscible liquids with more significant viscosity differences [23]. Hydrodynamic cavitation results from the fluid flowing past a restriction, expanding, and then bursting of cavities [20]. The lipid extraction process with hydrodynamic cavitation can reduce the extraction energy requirements so that they are lower [24], [25].

To increase energy efficiency, the extraction and transesterification processes of the hydrodynamic cavitation are carried out in one process (in-situ/direct transesterification) [26], [27]. The transesterification can be performed with or without a catalyst [12]. The transesterification reaction uses a catalyst to speed up the reaction by reducing the activation energy [28]. Catalyzed in-situ transesterification has been investigated by several researchers. In-situ transesterification with CaO/Hydrotalcite catalyst on *Nannochloropsis* sp. Using the microwave-assisted method with a biomass and methanol ratio of 1:10, produced a yield of 17.8% [29]. In-situ transesterification with H₂SO₄ catalyst on *Chlorella* sp. Using a batch reactor with a methanol and biomass ratio of 3:1 produced a yield of 96% [30]. There is still little research in the literature regarding the generation of biodiesel via direct transesterification based on hydrodynamic cavitation without a catalyst. So, this research aims to study the performance of the new method of catalytic hydrodynamic cavitation in the direct transesterification of *Nannochloropsis* sp. microalgae based on the biodiesel production reaction rate and its characterization, by comparing it with the catalytic stirring (mechanical stirring) method.

2. MATERIAL AND METHOD

2.1. Material

As received dry *Nannochloropsis* sp. microalgae in powder form with a lipid content of 16% g/g dry weight. It was obtained from the Brackish Water Cultivation Center Situbondo, East Java, Indonesia. N-hexane technical grade (density 0.66 kg/cc and normal boiling point=68 °C). Methanol technical grade (density 0.791 kg/cc and normal boiling point=64.7 °C) and sodium hydroxide (NaOH) of analytical grade were procured from CV Genera Labora. All chemicals have been used without any further treatment or purification.

2.2. Biodiesel production from catalytic direct transesterification processes using stirring method

The method was carried out by direct transesterification of 7.5 g of dry microalgae with a solvent mixture of 40 ml methanol, 90 ml n-hexane, and 0.015 g NaOH in an Erlenmeyer with varying times of 30, 60, 90, and 120 minutes. After the direct transesterification, the liquid and the solid were separated by precipitation and centrifugation. Centrifugation was carried out at 2,000 rpm for 5 minutes. To ensure that all the extraction results from the microalgae are taken, the microalgae solids are washed using methanol and n-hexane, and the washing result solution is added to the centrifugation result solution. The liquid phase obtained was separated by solvent evaporation at 68 °C until a constant weight result was obtained.

2.3. Biodiesel production from catalytic direct transesterification process using hydrodynamic cavitation method

The method was carried out by preparing an extractor reactor with hydrodynamic cavitation. The reactor was paired with a compressed air system to flow the sample through a cavitation generator. The direct transesterification process was conducted by introducing 7.5 g of microalgae, 40 ml of methanol, 90 ml of hexane, and 0.015 g NaOH into the sample chamber. The mixture was then put into the cavitation using a pressure driver with varying repetitions of 1, 2, 3, and 4 times. Each repetition assumes that the cavitation process time is 1 minute. The liquid (extract) and the solid phase formed during the process were separated using centrifugation at 2,000 rpm for 5 minutes. To ensure the complete extraction from the microalgae, the microalgae debris was washed using methanol and n-hexane, and then the washing solution was added to the extract. The liquid phase (extract) obtained was separated by evaporating the solvent at a temperature of 68 °C until a constant weight was obtained. An image of the equipment is illustrated in Figure 1.

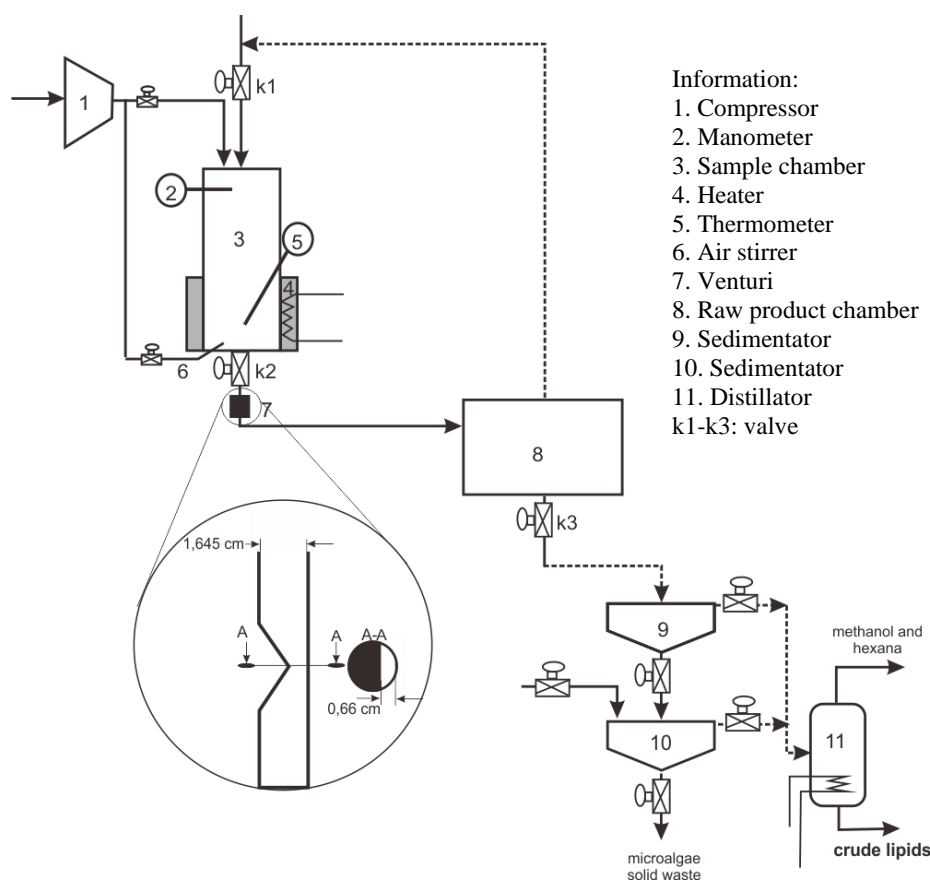


Figure 1. Equipment set up of hydrodynamic cavitation process

2.4. Biodiesel analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed to determine the chemical composition of the biodiesel. GC-MS (QP2010-SE, Shimadzu) with an RTx5MS capillary column (5% diphenyl-95% dimethylpolysiloxane cross bond) and quadrupole analyzer operated in electron impact mode of 70 eV was used for this analysis. ChemStation software was used to receive and process data. Identifying components involves comparing their mass spectra with the spectral data already in the database [31].

3. RESULTS AND DISCUSSION

3.1. Yield composition analysis

Figure 2 shows the GC-MS chromatogram of the extracted lipid from *Nannochloropsis* sp. microalgae produced from the catalytic hydrodynamic cavitation method were obtained at reaction times of

1 minute (Figure 2(a)), 2 minutes (Figure 2(b)), 3 minutes (Figure 2(c)), and 4 minutes (Figure 2(d)). Figure 2 shows that most extract components have almost the same chromatogram plots and are in the retention time range of 20-27 minutes for all time variables. Figure 2 reveals the presence of hexadecanoic acid, 2-hydroxy-1, and 3-propanediyl ester (diglyceride) as the predominant component. Stearic and palmitic acids are the predominant components among the fatty acids. Meanwhile, methyl 10-octadecanoate is the predominant component of fatty acid methyl ester. Meanwhile, the stirring trans-esterification process without cavitation hydrodynamics is carried out using mechanical stirrer electricity using the variable time as the independent variable.

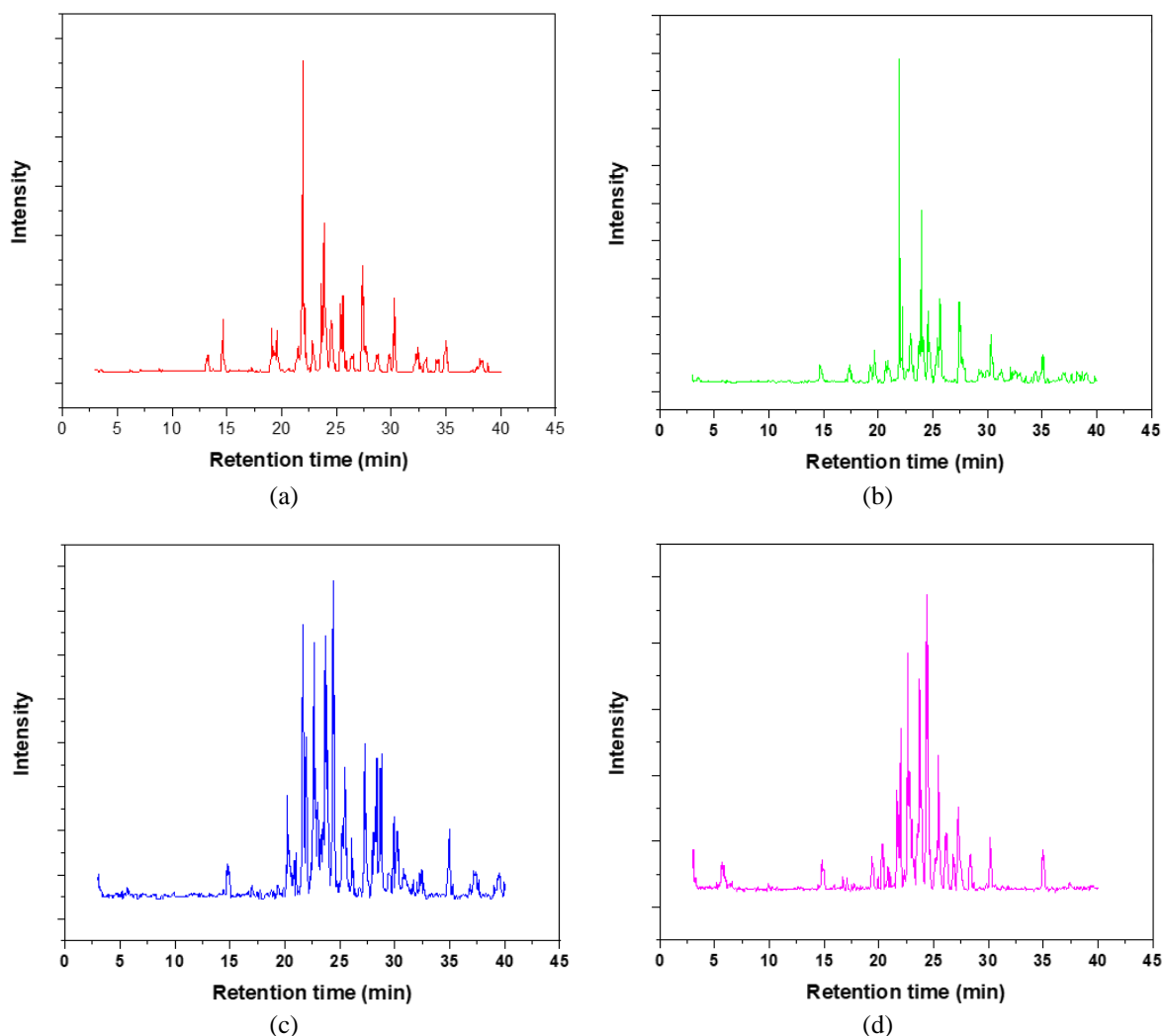


Figure 2. Chromatograms of the extracted lipid were obtained at reaction times of (a) 1 minute, (b) 2 minutes, (c) 3 minutes, and (d) 4 minutes using the catalytic hydrodynamic cavitation method

Figure 3 shows the GC-MS chromatogram of the extracted lipid from *Nannochloropsis* sp. microalgae extracts produced using the stirring method were obtained at different reaction times of 30 minutes (Figure 3(a)), 60 minutes (Figure 3(b)), 90 minutes (Figure 3(c)), and 120 minutes (Figure 3(d)). Figure 3 shows that most extract components have almost the same chromatogram plots and are in the retention times ranging between 20 and 27 minutes for all time variables. It is noted from Figure 3 that several substances appear in significant amounts, namely methyl palmitate and methyl 10-octadecanoic from the methyl ester group as well as palmitic acid, oleic acid, stearic acid, and hypogenic acid from the fatty acid group.

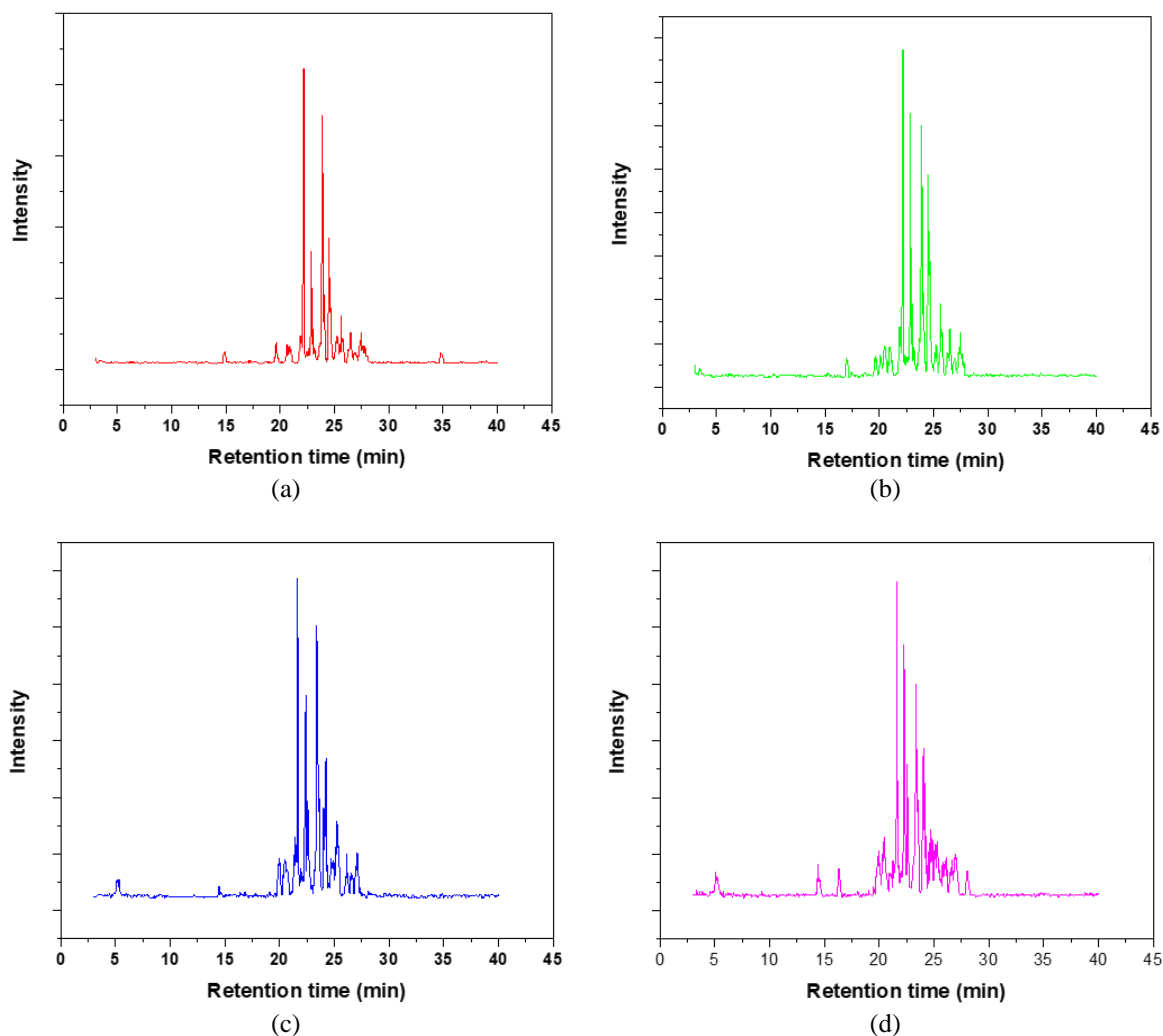


Figure 3. Chromatograms for the extracted lipid were obtained at different reaction times of (a) 30 minutes, (b) 60 minutes, (c) 90 minutes, and (d) 120 minutes using the stirring method

3.2. Comparison of direct transesterification time

Direct transesterification includes two processes, namely extraction and transesterification reactions. The extraction yield can be determined by measuring the total weight of the lipid and biodiesel mixture taken from dried microalgae, while the results of the transesterification reaction were identified by analysis using GC-MS.

The effect of different direct transesterification methods on the process time to obtain equivalent yield is presented in Figure 4. Figure 4(a) shows the effect of time on extract yield using hydrodynamic cavitation methods. It can be seen that the time significantly influences the yield of extract obtained. The longer the extraction time, the more yield will be obtained, with the highest yield of 11.73% at 4 minutes. Figure 4(b) shows the effect of reaction time on the yield of extract obtained from stirring methods. It shows that the longer the extraction time, the more yield it will get, with the highest yield at 120 minutes at 10.60%. This is because the longer the time, the greater the chance for collisions between the reactant molecules, thus increasing the product produced [32], [33]. However, because the transesterification reaction is reversible, there is an optimum time at which the highest yield is achieved [34]–[37]. Efavi *et al.* [38] investigate the effect of extraction time on the yield and properties of *Citrullus vulgaris* seeds, with varying times of 90, 120, and 150, resulting in an optimum extraction time of 120 minutes. The comparison of Figures 4(a) and 4(b) shows that the extraction process using hydrodynamic cavitation is much more effective than the stirring method, and it needs the lowest energy extraction [39].

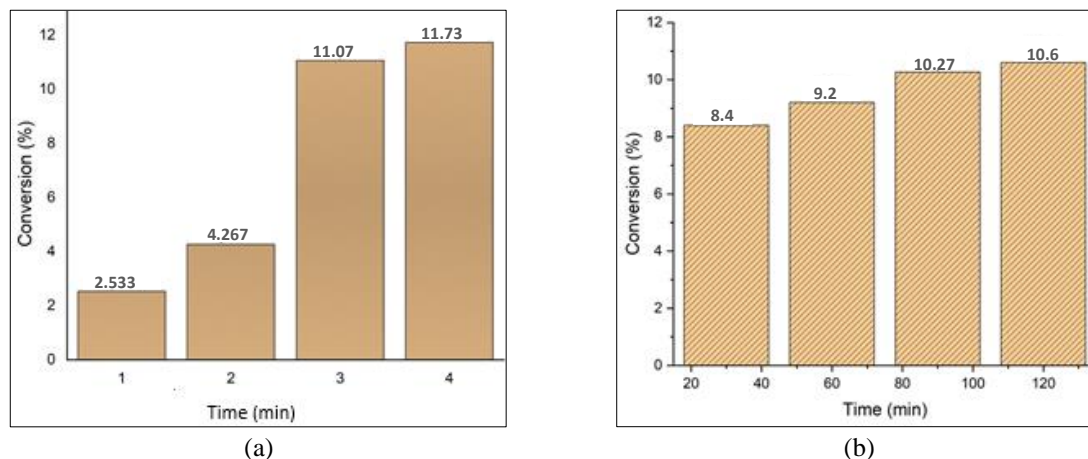


Figure 4. The effect of extraction time on the yield of the extracted lipid of *Nannochloropsis* sp. microalgae of (a) catalytic hydrodynamic cavitation method and (b) catalytic stirring method

Figure 5 shows the composition of the extracted lipid of *Nannochloropsis* sp. microalgae from the catalytic hydrodynamic cavitation method and the stirring method. Extract composition from *Nannochloropsis* sp. microalgae can be separated into three categories: fatty acid methyl ester (FAME), non-esterified fatty acid (NEFA), and glyceride. It is noted from Figure 5(a) that the percentage of FAME and glyceride decreases as time increases. Increasing the time increases the percentage of NEFA in the microalgae extract. The decrease in the percentage of glyceride (which includes triglyceride, diglyceride, and monoglyceride) indicates a transesterification process that converts glyceride into FAME and glycerol. Meanwhile, the increase in NEFA content as time increases may occur due to the free fatty acid (FFA) content in microalgae or the glyceride hydrolysis reaction that converts glyceride to fatty acid and glycerol. Lafont *et al.* [40] reported the possibility of triglycerides hydrolysis to produce fatty acids from milk as precursors to form deep eutectic solvents. From Figure 5(b), it can be seen that increasing the reaction (extraction) time from 30 to 120 minutes causes a decrease in the percentage of NEFA and glyceride, while FAME fluctuates with the optimum percentage at 90 minutes. Compared to Figures 5(a) and 5(b) shows a higher percentage of FAME and a lower percentage of glyceride. This illustrates that the stirring catalytic method with a longer time shows a more excellent conversion of glyceride to FAME than the catalytic hydrodynamic cavitation method. This indicates that time has an important role in the transesterification process. If we look at the percentage of NEFA, the stirring catalytic method produces a higher percentage of NEFA, but the longer the extraction time, the percentage of NEFA decreases. However, in the catalytic hydrodynamic method process, the percentage of NEFA increases with the increasing extraction time. In addition, the catalytic hydrodynamic method produces more glycerides than the stirring catalytic method.

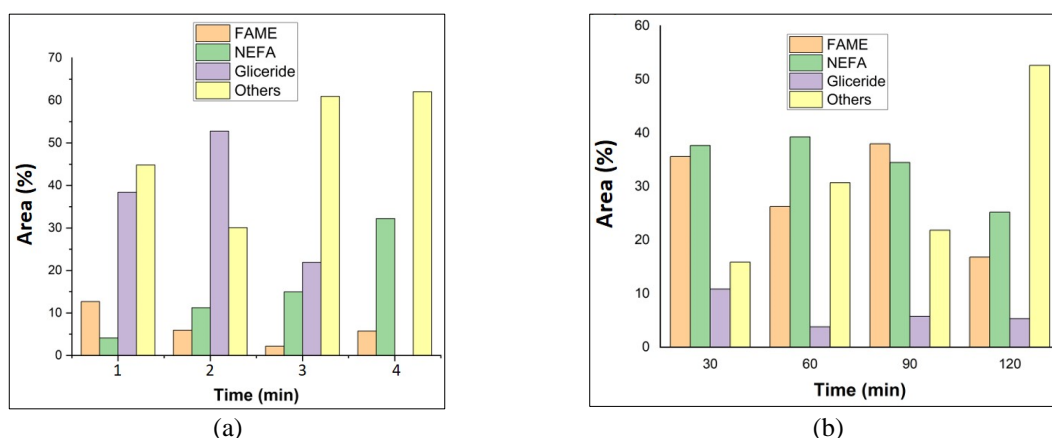


Figure 5. The composition of extracted lipid from *Nannochloropsis* sp. microalgae of (a) catalytic hydrodynamic cavitation method and (b) catalytic stirring method

Figure 6 shows the percentage of glyceride of *Nannochloropsis* sp. microalgae extract. Glycerides can be categorized into monoglycerides, diglycerides, and triglycerides. Figure 6 shows that triglyceride does not appear in the stirring catalytic method (Figure 6(b)), likewise, diglyceride appears in the stirring catalytic method in lower amounts than in the catalytic hydrodynamic cavitation method. Figure 6(a) shows that the catalytic hydrodynamic cavitation method produces higher diglyceride (19.8-30.65) and lower monoglyceride than the stirring catalytic method. This indicates that time has an important role in increasing the conversion of triglycerides into FAME.

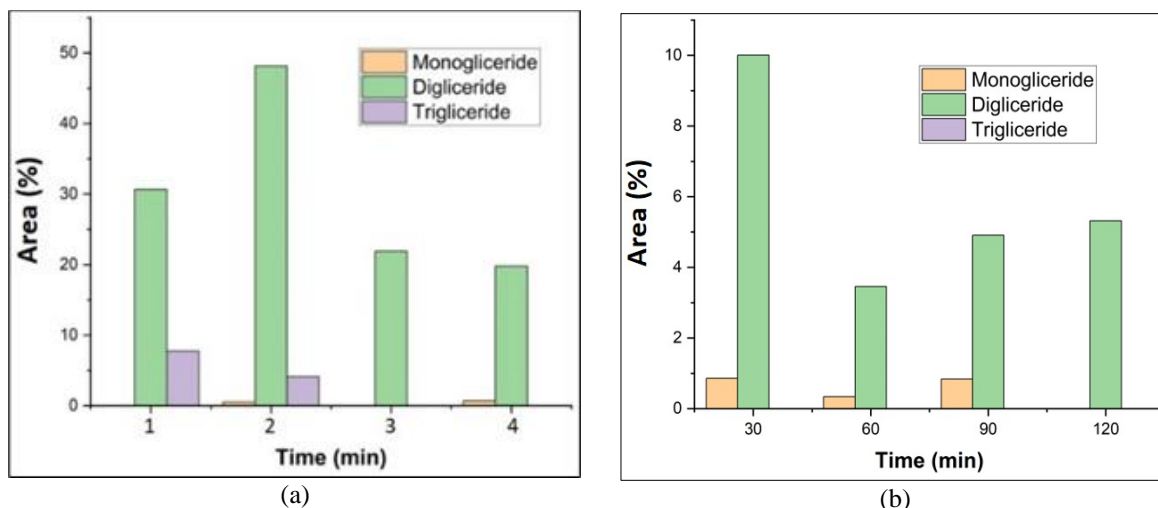


Figure 6. The percentage of glyceride of extracted lipid of (a) catalytic hydrodynamic cavitation method and (b) catalytic stirring method

3.3. Fatty acid methyl ester distribution

Table 1 shows the FAME distribution of the extracted lipid from the stirring method. Table 2 shows the FAME distribution in biodiesel from the hydrodynamic cavitation method. It can be seen from Tables 1 and 2 that esterification of *Nannochloropsis* sp. microalgae via stirring and hydrodynamic cavitation methods produces fatty acid methyl ester with chain length C10-C20. Concerning the chain length, it is observed that the predominant FAME in the stirring method is C16:0 and C18:1, while in the hydrodynamic cavitation method, are C12:0, C16:0, and C20:0 for the time of 1 minute, C12:0 and C16:0 for the time of 2 minutes, and C16:0 for the time of 3 and 4 minutes. Of these FAME, C18:1 (methyl oleate) is dominant in the stirring method, while C16:0 (methyl palmitate) is considered predominant in the hydrodynamic cavitation method. Oleic is a monounsaturated fatty acid beneficial to oxidation stability, while palmitic is a saturated fatty acid not advised for use as fuel in cold climates [41]. Tables 1 and 2 reveal that C16:0 (methyl palmitate) is significant in both methods. Tables 1 and 2 also show that C18:3 (methyl linoleate) appears in the stirring method and does not appear in the hydrodynamic cavitation method. Based on standard EN 14214:22, the maximum allowable amount of C18:3 (methyl linolenate) is 12% [42]. The high methyl linoleate content increases unsaturation, leading to poor oxidative stability [43].

Table 1. FAME distribution of the extracted lipid from the hydrodynamic cavitation method

Methyl ester	Molecular structure	Molecular weight	Molecular formula	Extraction time (minutes)			
				1	2	3	4
Laurate	12:0	214	C ₁₃ H ₂₆ O ₂	4.92	1.95	-	-
Myristate	14:0	242	C ₁₅ H ₃₀ O ₂	1.92	1.20	-	0.68
Palmitate	16:0	270	C ₁₇ H ₃₄ O ₂	2.14	1.80	1.54	3.68
Oleate	18:1	296	C ₁₉ H ₃₆ O ₂	0.72	-	0.63	-
Linolenate	18:3	292	C ₁₉ H ₃₂ O ₂	-	-	-	-
Stearate	18:0	298	C ₁₉ H ₃₈ O ₂	-	0.97	-	1.39
Arachate	20:0	326	C ₂₁ H ₄₂ O ₂	2.96	-	-	-
				12.66	5.92	2.17	5.75

Table 2. FAME distribution of the extracted lipid from the stirring method

Methyl ester	Molecular structure	Molecular weight	Molecular formula	Extraction time (minutes)			
				30	60	90	120
Laurate	12:0	214	C ₁₃ H ₂₆ O ₂	0.36	-	-	-
Myristate	14:0	242	C ₁₅ H ₃₀ O ₂	1.50	0.39	-	-
Palmitate	16:0	270	C ₁₇ H ₃₄ O ₂	12.41	9.57	9.30	6.62
Oleate	18:1	296	C ₁₉ H ₃₆ O ₂	14.34	9.76	9.42	9.10
Linolenate	18:3	292	C ₁₉ H ₃₂ O ₂	3.41	3.94	16.43	-
Stearate	18:0	298	C ₁₉ H ₃₈ O ₂	2.63	1.68	1.75	-
Arachate	20:0	326	C ₂₁ H ₄₂ O ₂	0.95	0.92	1.05	1.12

Notably, the FAME distributions produced by these two approaches differ significantly. Therefore, it is useful to create histograms that compare the FAME distributions of these two methods, considering the chain length and the degree of unsaturation, since these two have an impact on the primary properties of biodiesel, such as saponification number (SN), iodine value (IV), cetane number (CN), and higher heating value (HHV) [43], [44]. FAME can be separated into three groups short-chain FAME from C10-C14, medium-chain FAME from C16-C18, and long-chain FAME higher than C18. In contrast, according to the degree of unsaturation of the fatty acid that composes the ester, FAME can be separated into three groups: saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA). Using Tables 1 and 2, the histograms in Figures 7 and 8 are presented.

Figures 7 and 8 compare the FAME distribution of the two methods according to the chain length and the degree of unsaturation, respectively. From Figure 7, it can be observed that the medium-chain FAME is predominant. The catalytic hydrodynamic cavitation (Figure 7(a)) produces a lower percentage of medium-chain FAME in comparison to the stirring catalytic method (Figure 7(b)). Conversely, the catalytic hydrodynamic cavitation method (Figure 7(a)) produces higher short-chain FAME. The catalytic hydrodynamic cavitation and the stirring method provide an average chain length of 1.02 and 5.06, respectively.

It can also be observed from Figures 8(a) and 8(b) that the predominant FAME for both methods is SFA. In contrast, for the catalytic stirring method (Figure 8(b)), a substantial portion of monounsaturated fatty acid (MUFA) is produced. The portion of polyunsaturated fatty acid (PUFA) in both methods is small, under 10%, except for the catalytic stirring method (Figure 8(b)) at reaction times of 90 minutes, which produces 16.43% PUFA in the form of linolenic (C18:3). The catalytic hydrodynamic cavitation method is dominated by saturation fatty acid. In contrast, the catalytic stirring method has comparable saturation. The catalytic hydrodynamic cavitation and the stirring method provide an average degree of unsaturation of 0.0034 and 0.2849, respectively. This result reveals that the catalytic stirring method has a higher average chain length and degree of saturation than the catalytic hydrodynamic cavitation method.

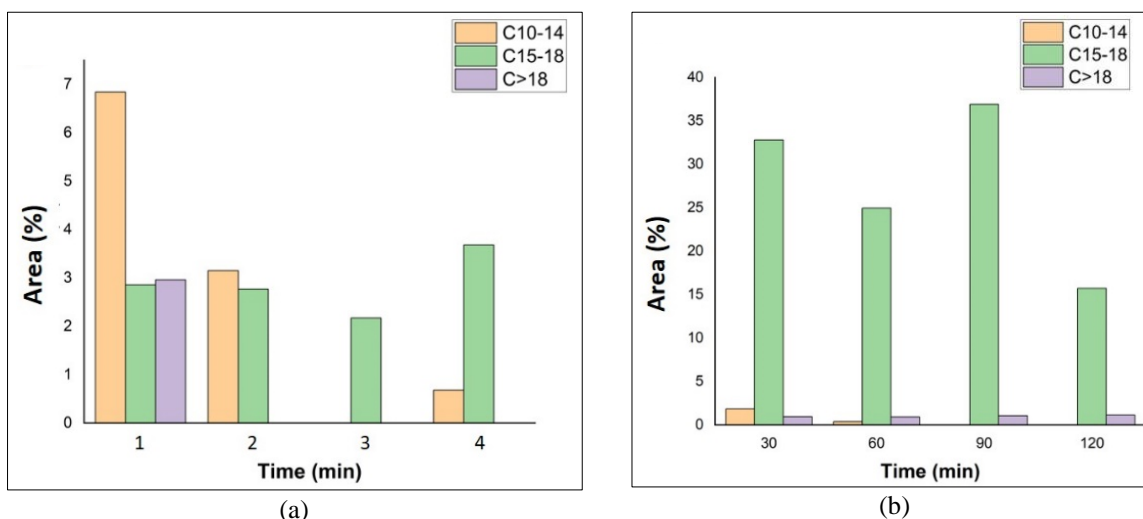


Figure 7. The chain length distribution of FAME of the extracted lipid from *Nannochloropsis* sp. microalgae of (a) the catalytic hydrodynamic cavitation method and (b) the catalytic stirring method

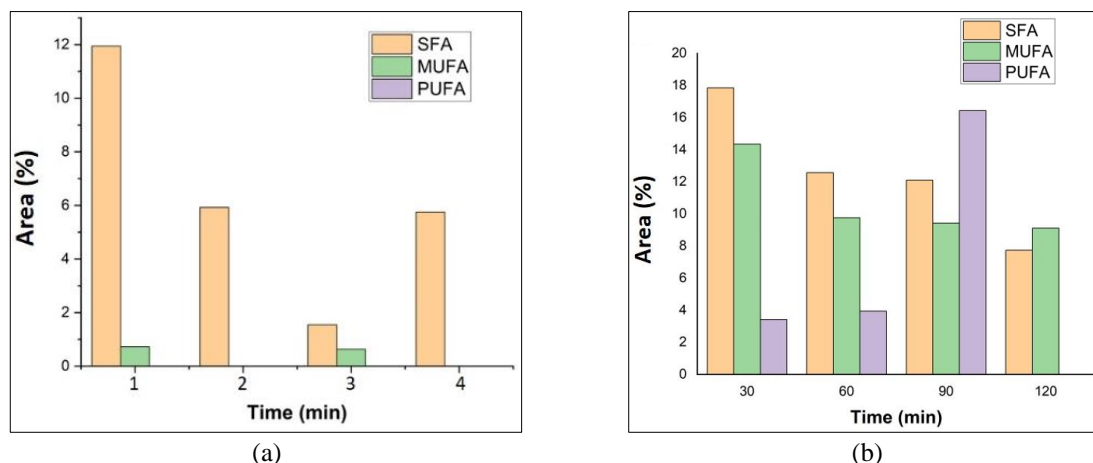


Figure 8. The degree of unsaturation distribution of FAME of the extracted lipid from *Nannochloropsis* sp. microalgae of (a) the catalytic hydrodynamic cavitation method and (b) the catalytic stirring method

4. CONCLUSION

Direct transesterification of *Nannochloropsis* sp. microalgae using the catalytic stirring method and catalytic hydrodynamic cavitation method has been conducted. Direct transesterification is carried out by performing extraction and transesterification processes simultaneously for cost-effectiveness. Longer reaction times result in higher yields in the catalytic stirring method. Meanwhile, the yield obtained from catalytic hydrodynamic cavitation also increases, along with the time. The catalytic hydrodynamic cavitation method produces a yield of extract that is slightly greater than the stirring catalytic method and takes a much shorter time. However, it produces a lower percentage of FAME. FAME composition from the catalytic hydrodynamic cavitation method is dominated by saturated fatty acid (palmitic), while FAME composition from the stirring catalytic method is dominated by monounsaturated fatty acid (oleic). The catalytic hydrodynamic cavitation method has a lower degree of unsaturation and average chain length than the catalytic stirring method. The stirring catalytic method performs better than the catalytic hydrodynamic cavitation method because it contains a higher amount of monosaturated fatty acid (oleic acid) in the respective FAME.

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AUTHOR CONTRIBUTIONS STATEMENT

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Martomo Setyawan	✓	✓		✓		✓		✓		✓		✓		
Siti Jamilatun		✓					✓			✓		✓		
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C : Conceptualization	I : Investigation	Vi : Visualization
M : Methodology	R : Resources	Su : Supervision
So : Software	D : Data Curation	P : Project administration
Va : Validation	O : Writing - Original Draft	Fu : Funding acquisition
Fo : Formal analysis	E : Writing - Review & Editing	

CONFLICT OF INTEREST STATEMENT

Authors state no conflict of interest.

DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author, [MS]. The data, which contain information that could compromise the privacy of research participants, are not publicly available due to certain restrictions.




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