Fatty Acid Composition of Three Diatom Species *Skeletonema costatum*, *Thalassiosira* sp. and *Chaetoceros gracilis*

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Abstract: The goal of this research work is to compare the lipid content of three diatom species (*Skeletonema costatum*, *Thalassiosira* sp., and *Chaetoceros gracilis*) and to identify the fatty acid composition of those species. Highest content of lipid was found in *Chaetoceros gracilis*, while the lowest was in *Skeletonema costatum*. The extracts of different solvents shown lipid content in some extent where chloroform produced higher lipid content compared to hexane. The highest Fatty Acids Methyl Esters (FAME) found in *Chaetoceros gracilis* was methyl palmitic (C\(_{16:0}\)), 33.29 % extraction with chloroform as solvent and methyl palmitoleic (C\(_{16:1}\)), 49.42 % extraction with hexane as solvent. The highest Fatty Acids Methyl Esters (FAME) found in *Skeletonema costatum* was methyl palmitoleic (C\(_{16:1}\)), 31.15 % extraction with hexane as solvent and methyl myristic (C\(_{14:0}\)), 41.46 % extraction with chloroform as solvent. The highest Fatty Acids Methyl Esters (FAME) found in *Thalassiosira* sp. was methyl palmitic (C\(_{16:0}\)), 34.17 % extraction with chloroform solvent and methyl palmitoleic (C\(_{16:1}\)), 44.72 % extraction with hexane solvent.

Keywords: Lipid, FAME, *Skeletonema costatum*, *Thalassiosira* sp., *Chaetoceros gracilis*, Hexane, Chloroform

1. Introduction

The demand for fossil fuel in Indonesia is increasing in conjunction with its national growth and development. In 2011, the demand for fossil fuel is 56 million kiloliter per year and has increased...
for 4% in average. While the supplies can only provide about 41 million kiloliter, which consists of 12 million kiloliter gasoline, 18.3 million kiloliter diesel fuel, 7 million kiloliter kerosene and 3.3 million kiloliter of aviation fuel. This imbalance between demand and supply can end up in energy (fuel) crisis. Thus, it is very important to find an alternative fuel source, such as bio fuel. Such activities have already started using crude palm oil (CPO), corn, soybean, canola, jatropha, coconut, and microalgae. The production capacity of corn is 172 liters per hectare, soybean is 446 liters per hectare, beans is 2689 liters per hectare, oil palm is 5950 liters per hectare and microalgae is 58700 liters per hectare (Chisti, 2007).

One of the most potential and environmentally friendly materials is microalgae. Microalgae can be massively extracted and converted into biofuel. With this material, biofuel can quickly be produced and with an environmental friendly process. Microalgae have the capability to absorb CO$_2$ generated by vehicles, industrial, respiratory and decomposition processes. Indonesia has vast water areas that highly potential for microalgae culture to produce biofuel.

Researches have already been conducted previously regarding microalgae utilization for biofuel, including both its production analysis and fatty acid analysis. This also includes production improvement from microalgae growth and lipid extraction process (Chisti, 2007; Benemann, 2008; Hu et al., 2008), fatty acid analysis of several microalgae species (Pratoomyot et al., 2005). Biodiesel is alkyl esters compound created from transesterification process between triglyceride and methanol, or esterification of free fatty acids (FFA) with methanol that gives methyl ester compound and water. Thus to understand the proportion of biodiesel fuel compound, fatty acid identification is needed.

2. Materials and Methods

2.1. Time and Location

This research was conducted in May-August of 2011 at Surfactant and Bioenergy Research Center (SBRC) Laboratory, Institute of Research and Community Service (LPPM), Bogor Agricultural University (IPB). Sample analysis was conducted at Integrated Laboratory Center in State Islamic University Syarif Hidayatullah, Jakarta.

2.2. Microalgae Samples Collection

Microalgae samples that are in growth phase were collected from culturing pond in Research Center for Marine Aquaculture of Marine and Fishery Ministry in Bali. Harvesting was conducted by adding 150 ppm NaOH to make the microalgae settled at the bottom of the pond. Afterward, fresh water is added to the medium to create a solution with ratio of 3:1 of fresh water: microalgae biomass, to lower the salinity and facilitate microalgae filtering. Filtering was conducted using satin cloth 3 µm
(smaller than microalgae size). Next, the microalgae were dried using sunlight for approximately 6 hours and by putting them in the oven for 3 hours at 400 °C temperatures. The dried microalgae were grinded using masher and blender until microalgae turned into powder.

2.3. Soxhlet Extraction

15 grams of microalgae samples was weighed and wrapped with filtering paper and fat free cotton. Samples were then extracted with 200 mL n-hexane solution for 6-7 hours inside soxhlet tube. Lipid extract were then evaporated using rotary evaporator and dried inside an oven for an hour with 50-60°C temperature. And lastly, it was weighed to find the lipid content.

2.4. Esterification

The purpose of esterification is to lower fatty acid evaporation temperature by changing the lipid functional group into esters which was easier with Chromatography-Mass Spectrometry (GC-MS) analysis. 0.5 – 1 gram of extracted lipid samples were disaponificated using 4.5 mL 0.5M of NaOH solution. Then esterification was performed by mixing triglyceride with BF3 methanol to create fatty acids methyl esters (biodiesel fuel) with BF3 acted as catalyst. The catalyst was used to increase reaction and yield rate. This process was conducted at 60°C using vortex mixing to increase reactant collision frequency. Stirred and heated for 15 minutes. The solution was then set aside to form two layers. The top layer was separated by centrifugation and purified by adding Na2SO4 to remove the water. The resulted mixture of this process was put inside a vial to be analyzed using GC-MS instrument.

2.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed by using Shimadzu QP2010 chromatography with silica column DB-5 ms (length 30 m, 0.25 mm intra diameter, and 0.25 µm film layer thickness) and helium as carrier gas. Gas Chromatography has a detection limit of 0.001 ppb. Split injection method with 1:200 ratio was used for gas chromatographic separation. The chromatography programming temperature is as following: initial temperature was set to 80°C for two minutes, then increase at 100 °C per minute to 210°C, and holds for 1 minute, and then increase at 60 °C per minutes to 280°C and holds for 5 minute. Mass Spectrometer analysis condition: potential ionization / electron energy is 70eV, ion source temperature is 250°C and interface temperature set to 280°C. Full mass data was recorded between 50-400 Dalton per second. GC retention time is 0-32.67 minutes. Data was recorded and analyzed using GC-MS Real Time Analysis and GC-MS Postrun Analysis software.
2.6. Fatty Acid Identification

Diatom fatty acid identification was conducted using gas chromatography and gas chromatography-mass spectrometry. Methyl esters identification was conducted by comparing the mass spectra with the literature data. Carbon number of the methyl esters compound was determined by calculating molecule weight that appeared in mass spectra. Fatty acid methyl esters characteristic is most powerful at m/z = 74 which is the basic peak of methyl esters straight chain. Widest peak in each group represented the fragment C\(_n\)H\(_{2n-1}\)O\(_2\) and m/z = 14 (n -2) + 74, or in a simpler way can be stated as:

\[
C_x = \frac{m-74}{14} + 2
\]

where:

\(x\) = carbon number (FAME)

\(m\) = molecule mass that appeared in the peak of mass spectra.

14 = molecule mass of CH\(_2\)

2.7. Data Analysis

Data analysis was done by comparing lipid content and fatty acids components among the three diatom species (*Chaetoceros gracilis*, *Skeletonema costatum*, and *Thalassiosira* sp.). That comparison was carried out using tables and figures. Based on the result, conclusion can be drawn by conducting literature study.

3. Results and Discussion

3.1. Microalgae Extraction

In this research, the extracted materials were grinded to widen the tangent surface between solvent and extracted material. As for extraction period, the process was stopped when the solvent inside the thimble turned colorless as a sign that lipid within the microalgae has been extracted completely. Since the extracted lipid was non-polar, the solvent used must has the same polarity as the lipid and oil, so the lipid can be dissolved. The lipid created was gross lipid which consists of natural lipid and polar lipid. Natural lipid consists of triglyceride, waxes ester, hydrocarbon, free fatty acids and sterol. Whereas, polar lipid consists of phospholipids, glycolipid, chlorophyll, and carotenoids (Winaryo, 2009).
3.2. Diatom Lipid Content Percentage

The result showed that the three diatom species that have experienced lipid extraction have different lipid content. Different solvents also give different lipid content. Lipid content data showed that Chaetoceros gracilis has the highest lipid content, which was 10.17% when extracted using n-hexane as solvent and 12.36% when extracted using chloroform. Skeletonema costatum has the lowest lipid content of 6.45% when extracted using n-hexane as solvent and 9.25% when extracted using chloroform. The lipid content of Thalassiosira sp. is 7.80% % when extracted using n-hexane as solvent and 10.43% when extracted using chloroform.

Lipid of microalgae tends to be inversely proportional to growth rate, while several environmental factors also play a role in affecting fatty acid relative proportion and lipid total content (Borowitzka, 1987). The higher the growth rate, the less amount of lipid microalgae will have. It is confirmed that with a slow growth rate, the energy needed to growth was converted into lipid production as food storage.

Based on the lipid percentage, hexane and chloroform solvents gave different results, where chloroform dissolved more microalgae lipid. When microalgae were extracted, all lipids in microalgae would be included, so several algae will give a dark green extraction product (Winaryo, 2009). This research extraction showed that chloroform extracted algae showed dark green extraction product. This is caused by the nature of the chloroform which was more polar than n-hexane, so the lipid polar component, such as chlorophyll and phospholipid will also be extracted (Winaryo, 2009). The result of fatty acid percentage from the three species is shown in Table 1.

Table 1. Fatty acid percentage of the three species of diatom extracted with n-hexane and chloroform

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Repetition</th>
<th>Skeletonema costatum (%)</th>
<th>Thalassiosira sp. (%)</th>
<th>Chaetoceros gracilis (%)</th>
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<td>3</td>
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<td>8.14</td>
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<tr>
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<td>7.80</td>
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<td></td>
<td>St. Dev</td>
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<td>Chloroform</td>
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<td>10.58</td>
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<td></td>
<td>St. Dev</td>
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</tr>
</tbody>
</table>

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3.3. Microalgae Fatty Acids Methyl Esters (FAME) Identification

Microalgae fatty acids methyl esters identification was conducted by observing fatty acids methyl esters compounds chromatogram, which has been recorded for almost 33 minutes. Fatty acids methyl esters characteristic that appeared on the mass spectra was characterized as mass to charge ratio (m/z) 74. Molecular peak is also needed to be observed to determine the carbon number of fatty acids methyl esters compounds.

Most abundant fatty acids methyl esters detected within the diatoms are methyl palmitic (C\textsubscript{16:0}), methyl myristic (C\textsubscript{14:0}), and methyl palmitoleic (C\textsubscript{16:1}). Diatom’s methyl palmitic (C\textsubscript{16:0}) characteristics was based on the base peak (m/z) 270 (Fig. 1), and then its mass spectra was identified. Unlike the saturated fatty acids methyl esters, for methyl palmitoleic (C\textsubscript{16:1}) the detection was based on base peak (m/z) 268 (Fig. 2). Molecule mass reduction had occurred from 270 in methyl palmitic (C\textsubscript{16:0}) to 268 in methyl palmitoleic (C\textsubscript{16:1}). This indicated that there was an additional double bond, where every double bond added, there was 2 atom molecule mass reduction from the previous saturated fatty acid mass (Christie, 2012). Dissimilarity also occurred in molecule ion that dominated mass spectra, in saturated fatty acid the base peak was characterized with mass to charge ratio (m/z) 74, while the singular double bond unsaturated fatty acid has base peak with mass to charge ratio (m/z) 55 (Christie, 2012).

![Mass spectra of methyl palmitic compound (C\textsubscript{16:0}) in diatom](image)

**Fig. 1.** Mass spectra of methyl palmitic compound (C\textsubscript{16:0}) in diatom
3.4. Fatty Acids Methyl Esters (FAME) of Chaetoceros Gracilis

Fatty acids methyl esters characteristics of *Chaetoceros gracilis* that was extracted using chloroform were between C$_{14}$ to C$_{24}$ (Fig. 3). These methyl esters consist of the fatty acids groups: SAFA (Saturated fatty acids) 63.05 %, MUFA (Monounsaturated fatty acids) 34.01 %, and PUFA (Polyunsaturated fatty acids) 2.94%. SAFA fatty acids methyl esters, consist of methyl myristic (C$_{14}$) 20.66 %, methyl pentadecanoic (C$_{15}$) 1.61 %, methyl palmitic (C$_{16}$) 33.29 %, methyl stearic (C$_{18}$) 4.64 %, methyl arachidic (C$_{20}$) 0.30 %, methyl behenic (C$_{22}$) 0.43 %, and methyl lignoceric (C$_{24}$) 0.69 %, with the largest amount of SAFA content are C$_{16}$ and C$_{14}$. MUFA fatty acids methyl esters, consists of methyl palmitoleic (C$_{16:1}$) 31.00 %, and methyl oleic (C$_{18:1}$) 2.63 %.

Fatty acids methyl esters characteristics of *Chaetoceros gracilis* that was extracted using hexane were between C$_{13}$ to C$_{24}$ (Fig. 4). These methyl esters consist of two fatty acids SAFA 44.44
%, and MUFA 56.42 %. SAFA methyl esters consists of methyl tridecyclic (C_{13}) 0.32 %, methyl myristic (C_{14}) 10.39 %, methyl pentadecylic (C_{15}) 3.57 %, methyl palmitic (C_{16}) 15.44 %, methyl margaric (C_{17}) 0.71 %, methyl stearic (C_{18}) 9.09 %, methyl arachidic (C_{20}) 0.69 %, methyl behenic (C_{22}) 1.83 %, and methyl lignoceric (C_{24}) 1.52 %, with the largest SAFA content were C_{16} and C_{14}. MUFA fatty acid methyl esters consists of methyl palmitoleic (C_{16:1}) 49.42 %, and methyl oleic (C_{18:1}) 6.14 %.

Based on Renaud et al. (2002) in Hu et al. (2008), Chaetoceros sp. fatty acid content consists of myristic acid (C_{14}) 23.60 %, palmitic acid (C_{16}) 9.20 %, palmitoleic acid (C_{16:1}) 36.50 %, hexadecadienoic acid (C_{16:2}) 6.9 %, hexadecatrienoic acid (C_{16:3}) 2.60 %, margaric acid (C_{17}) 2 %, and oleic acid (C_{18:1}) 3 %. The most dominant fatty acids in Chaetoceros gracilis harvested during stationery phase were palmitic acid (C_{16}) 32.83 %, myristic acid (C_{14}) 20.32 %, and oleic acid (C_{18:1}) 31.05 % (Pratiwi et al., 2009).

![Fig. 4: Total of fatty acid methyl esters ionic current of Chaetoceros gracilis extracted with hexane](image)

Microalgae extracted using hexane solvent showed different result, compared to microalgae extracted using chloroform, where methyl tridecyclic and methyl margaric were detected when using hexane as solvent, but absent when extracted using chloroform. Different results also occurred with SAFA and MUFA content of Chaetoceros gracilis, when using chloroform the SAFA and MUFA content were 63.05 % and 34.01 %, and 44.44 % and 56.42 % when using hexane solvent.

### 3.5. Fatty Acids Methyl Esters (FAME) of Skeletonema costatum

Fatty acids methyl esters characteristics of Skeletonema costatum that was extracted using chloroform were between C_{13} to C_{24} (Fig. 5). These methyl esters consist of the fatty acids groups:
SAFA 68.31 %, MUFA 29.59 %, and PUFA 2.10 %. SAFA fatty acids methyl esters, consists of methyl tridecyl (C$_{13}$) 0.52 %, methyl myristic (C$_{14}$) 41.46 %, methyl pentadecyl (C$_{15}$) 2.27 %, methyl palmitic (C$_{16}$) 22.36 %, methyl margaric (C$_{17}$) 0.28 %, methyl stearic (C$_{18}$) 0.88 %, methyl behenic (C$_{22}$) 0.16 %, and methyl lignoceric (C$_{24}$) 0.38 %, with the largest amount of SAFA content are C$_{14}$ and C$_{16}$. MUFA fatty acids methyl esters, consists methyl palmitoleic (C$_{16:1}$) 26.68 %, and methyl oleic (C$_{18:1}$) 2.91 %. PUFA fatty acids methyl esters, consists of methyl hexadecadienoic (C$_{16:2}$) 2.10 %.

Fatty acids methyl esters characteristics of Skeletonema costatum that was extracted using hexane were between C$_{10}$ to C$_{25}$ (Fig. 6). These methyl esters consist of two fatty acids SAFA 63.40 % and MUFA 36.60 %. SAFA methyl esters consists of methyl capric (C$_{10}$) 0.40 %, methyl lauric (C$_{12}$) 1.97 %, methyl tridecyl (C$_{13}$) 4.20 %, methyl myristic (C$_{14}$) 14.37 %, methyl pentadecyl (C$_{15}$) 11.38 %, methyl palmitic (C$_{16}$) 12.83 %, methyl margaric (C$_{17}$) 1.65 %, methyl stearic (C$_{18}$) 6.63 %, methyl arachidic (C$_{20}$) 0.92 %, methyl behenic (C$_{22}$) 2.17 %, methyl tricocyclic (C$_{23}$) 0.38 %, methyl lignoceric (C$_{24}$) 6.01 %, and methyl pentacocyclic (C$_{25}$) 0.49 %, with the largest SAFA content were C$_{14}$ and C$_{16}$. MUFA fatty acid methyl esters consist of methyl palmitoleic (C$_{16:1}$) 31.15 %, methyl oleic (C$_{18:1}$) 4.73 %, and methyl nervonic (C$_{24:1}$) 0.72 %.

**Fig. 5:** Total of fatty acid methyl esters ionic current of Skeletonema costatum extracted with chloroform
Winaryo (2009) stated that the most dominant fatty acid content of *Skeletonema costatum* were palmitic acid (C₁₆) 16.50 %, myristic acid (C₁₄) 16.50 %, and arachidic acid (C₂₀:₅) 40.70 %. And based on the research done by Berge (1995), the main content (PUFA) of *Skeletonema costatum* are C₁₆:₁, C₁₆:₂, C₁₆:₃, and C₂₀:₅.

The hexane extracts of microalgae gave differing result from the one that was extracted using chloroform, in the former methyl capric, lauric, arachidic, tricocyclic, and pentacocyclic were found, but not in the one using chloroform. Different result also occurred in SAFA, MUFA, and PUFA, where *Skeletonema costatum* with chloroform has SAFA, MUFA, and PUFA has 68.31 %, 29.59 %, and 2.10 % content respectively, while using hexane the SAFA and MUFA content are 63.40 % and 36.60 %, with no amount of PUFA detected.

3.6. Fatty Acids Methyl Esters (FAME) of *Thalassiosira* sp.

Fatty acids methyl esters characteristics of *Thalassiosira* sp that was extracted using choloform were between C₁₄ to C₂₄ (Fig. 7). These methyl esters consist of the fatty acids groups: SAFA (Saturated fatty acids) 67.22 %, MUFA 31.89 %, and PUFA 0.89 %. SAFA fatty acids methyl esters, consists of methyl myristic (C₁₄) 20.93 %, methyl pentadecylic (C₁₅) 9.13 %, methyl palmitic (C₁₆) 34.17 %, methyl marginic (C₁₇) 0.96 %, methyl stearic (C₁₈) 0.80 %, and methyl lignoceric (C₂₄) 1.23 %, with the largest amount of SAFA content are C₁₄ and C₁₆. MUFA fatty acids methyl esters, consists of palmitoleic (C₁₆:₁) 31.89%. PUFA fatty acids methyl esters, consist of methyl hexadecadienoic (C₁₆:₂) 0.89 %.
Fig. 7: Total of fatty acid methyl esters ionic current of *Thalassiosira* sp extracted with chloroform

Fatty acids methyl esters characteristics of *Thalassiosira* sp that was extracted using hexane were between C<sub>12</sub> to C<sub>25</sub> (Fig. 8). These methyl esters consist of the fatty acids groups: SAFA 50.43 %, MUFA 48.38 %, and PUFA 1.19 %.

Fig. 8: Total of fatty acid methyl esters ionic current of *Thalassiosira* sp extracted with hexane

SAFA fatty acid methyl esters compounds consist of methyl lauric (C<sub>12</sub>) 0.36 %, methyl tridecylic (C<sub>13</sub>) 0.52 %, methyl myristic (C<sub>14</sub>) 11.15 %, methyl pentadecylic (C<sub>15</sub>) 13.96 %, methyl palmitic (C<sub>16</sub>) 16.64 %, methyl margaric (C<sub>17</sub>) 2.00 %, methyl stearic (C<sub>18</sub>) 1.88 %, methyl behenic (C<sub>22</sub>) 0.49 %, methyl lignoceric (C<sub>24</sub>) 3.26 %, and methyl pentacocyclic (C<sub>25</sub>) 0.17 %, thus the biggest SAFA compounds were C<sub>15</sub> and C<sub>16</sub>. MUFA fatty acid methyl esters compounds consist of methyl myristoleic (C<sub>14:1</sub>) 0.38 %, methyl pentadecenoic (C<sub>15:1</sub>) 0.62 %, methyl palmitoleic (C<sub>16:1</sub>) 44.72 %, and methyl oleic (C<sub>18:1</sub>) 2.66 %. Based on the research by Pratoomyot *et al.* (2005), the most dominant
fatty acid compounds in *Thalassiosira* sp. that were harvested in stationery phase were palmitic acid (C\textsubscript{16}) is 20.67 %, myristic acid (C\textsubscript{14}) 6.37 %, and palmitoleic acid (C\textsubscript{16:1}) 42.02 %.

The hexane extracts of *Thalassiosira* sp. gave differing result from the one that was extracted using chloroform, where the one that was extracted using hexane, methyl lauric, tridecylic, behenic, and pentacocylic were found, but not in the one using chloroform. Different result also occurred in SAFA (Saturated fatty acids), MUFA (Monounsaturated fatty acids), and PUFA (Polyunsaturated fatty acids), where *Thalassiosira* sp. with chloroform has SAFA, MUFA, and PUFA has 67.22 %, 31.89 %, and 0.89 % content respectively, and 50.43 %, 48.38 %, and 1.19 % when using hexane.

### 3.7 SAFA, MUFA, and PUFA Contents in Each of Three Diatom Species

SAFA contents in *Skeletonema costatum* were between 63.40% and 68.31%, MUFA contents were 29.59 % to 36.60 % and PUFA contents were 0 % to 2.1 %. SAFA contents in *Chaetoceros gracilis* were between 44.44 % and 63.05 %, MUFA content was 34.01% to 52.92% and PUFA contents were 2.64 % to 2.94 %. SAFA content in *Thalassiosira* sp. were between 50.43% and 67.22%. MUFA content was 31.89 %to 48.38 % and PUFA contents were 0.89 % to 1.19 %.

Generally, SAFA is the most dominant in all three diatoms, this is consistent with the research of Tonon et al. (2002) in Pratiwi et al. (2009), where SAFA is the most dominant fatty acid compared to MUFA and PUFA. SAFA, MUFA, and PUFA contents can be altered by changing the environment condition and culture medium (Mansour et al., 2003; Rousch et al., 2003). Low temperature can increase the synthesis of unsaturated fatty acid, since in low temperature oxygen availability will rise. With the high amount of oxygen, enzyme process in desaturation reaction will be accelerated (Chen and Jiang, 2000).

### 3.8. Comparison of Fatty Acids Methyl Esters (FAME) in Each Three Species

Fatty Acids Methyl Esters (FAME) that were detected within the three species ranged between C\textsubscript{10} to C\textsubscript{25} (Table 2). The most dominant compounds detected were methyl myristic (C\textsubscript{14:0}), methyl palmitic (C\textsubscript{16:0}), and methyl palmitoleic (C\textsubscript{16:1}), while the least was methyl undecyclic (C\textsubscript{11}). The most dominant FAME compounds extracted using chloroform from the three species were methyl esters palmitic (C\textsubscript{16}) in *Chaetoceros gracilis* and *Thalassiosira* sp., and methyl esters myristic (C\textsubscript{14}) in *Skeletonema costatum*. While the most dominant FAME compounds extracted using hexane was methyl esters palmitoleic (C\textsubscript{16:1}) in all three species.

According to Borowitzka and Borowitzka (1988), the major contents of *Bacillariophyceae* (diatom) fatty acids were palmitic acid (C\textsubscript{16:0}), hexadecenoic (C\textsubscript{16:1}) and polynoic (C\textsubscript{20}), and the minor content was linoleic acid (C\textsubscript{20}). Pratoomyot (2005) also stated that the major contents of
Bacillariophyceae (diatom) fatty acids were C\textsubscript{16:1}, C\textsubscript{16:0}, and C\textsubscript{20:5}. These facts are consistent to above FAME data which stated that palmitic acid (C\textsubscript{16:0}) and palmitoleic (C\textsubscript{16:1}) are the major contents of Bacillariophyceae (diatom) fatty acids.

Table 2. Composition of fatty acid methyl esters (FAME) from diatom (percentage of total fatty acids)

<table>
<thead>
<tr>
<th>FAME</th>
<th>Chaetoceros gracilis</th>
<th>Skeletonema costatum</th>
<th>Thalassiosira sp.</th>
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</thead>
<tbody>
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<td></td>
<td>Chloroform</td>
<td>Hexane</td>
<td>Chloroform</td>
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Triglyceride was produced by specific strain/species that eventually controlled by genetic composition by that organism individual. Microalgae produced a small amount of triglyceride under
optimum growth or advantageous environmental condition (Hu et al., 2008). Synthesis and high triglyceride accumulation and significant change in fatty acids composition, occurred when microalgae experienced a stressful condition, both from chemical or physical stimulation. Main chemical stimulation is nutrient impoverishment, while main physical stimulation is temperature and light intensity. Microalgae growth phase also affect triglyceride and microalgae fatty acid composition.

The most influential nutrient to lipid metabolisms was nitrogen, nitrogen limitation caused triglyceride accumulation (Hu et al., 2008). Silicone is as important as nitrogen in affecting lipid metabolisms. When lack of silicon occurred, Saturated Fatty Acids (SAFA) and Monounsaturated Fatty Acids (MUFA) increase (Hu et al., 2008). Phosphore limitation can increase triglyceride content in Chaetoceros sp. (Bacillariophyceae), I. galbana (Prymnesiophyceae), but decrease triglyceride in Nannochlorosis atomus (Chlorophyta) and Tetraselmis sp. (Prasinophyceae) (Hu et al., 2008).

Temperature and light intensity also affected microalgae fatty acid composition. Temperature drop will increase unsaturated fatty acid and temperature rise will increase saturated fatty acid in microalgae. Low light intensity will induce lipid polar forming, especially the one that connected with the chloroplast, and high light intensity will increase the amount of neutral lipid, especially triglyceride (Hu et al., 2008).

3.9. The Effect of Fatty Acids Methyl Esters (FAME) to Biodiesel

Chemical composition between biodiesel fuel and fossil diesel fuel are very different. Fossil diesel fuel usually has 30-35% aromatic hydrocarbon, 65-70% paraffin, and olefins traces that mostly within the C10 and C16 range. While biodiesel fuel has C16 and C18 fatty acid methyl esters with one to three double bond per molecule (Mittelbach and Remschmidt, 2006).

Some biodiesel fuel parameter such as density, cetane number and sulphure content are influenced by oil type that was used. Density difference is affected by fatty acid composition and purity of the material. Density will raise along with lower carbon chain length and higher number of double bond in the fatty acid, so the more unsaturated the oil contains, the higher density is obtained (Mittelbach and Remschmidt, 2006). As in density, biodiesel fuel cetane number is affected by fatty acid methyl esters composition. The more unsaturated the methyl esters of the oil, the lower cetane number is obtained.

The lower the cetane number, the lower the quality of flame. Along with unsaturated fatty acid, length of carbon chain also influenced cetane number (Mittelbach and Remschmidt, 2006). Research results from Gorpinath et al. (2009) stated that stearat acid (C18:0) has cetane number of 85.9, palmitate acid (C16:0) 76.6, miristate acid (C14:0) 66.9, laurate acid (C12:0) 61.1, oleat acid (C18:0) 56.9, linoleat acid (C18:2) 39.2, and linolenat acid (C18:3) 28.
Based on the results of this research, fatty acids methyl esters content within those three species can affected the density and cetane number of the biodiesel produced. The highest SAFA content was found in *Skeletonema costatum*, thus it can be concluded that *Skeletonema costatum* has lower biodiesel density, while higher *Chaetoceros gracilis* has MUFA and PUFA content, thus it has higher biodiesel density. In contrast to density, cetane number has parallel relationship with SAFA content, higher SAFA content, the higher cetane number get. It can be concluded that *Skeletonema costatum* has a higher cetane number amongst the three species, while *Chaetoceros gracilis* has the lowest number.

4. Conclusions

The highest lipid content was found in *Chaetoceros gracilis* extracted with both chloroform and hexane solvent, while the lowest was found in *Skeletonema costatum*. Different extraction solvents also gave different lipid content, where chloroform gives higher lipid content compared to hexane. The highest Fatty Acids Methyl Esters (FAME) content found in *Chaetoceros gracilis* was methyl palmitic (C\(_{16:0}\)) extracted by chloroform and methyl palmitoleic (C\(_{16:1}\)) extracted by hexane. The highest FAME found in *Skeletonema costatum* was methyl palmitoleic (C\(_{16:1}\)) extracted by hexane and methyl myristic (C\(_{14:0}\)) extracted by chloroform. The highest FAME found in *Thalassiosira* sp was methyl palmitic (C\(_{16:0}\)) extracted by chloroform and methyl palmitoleic (C\(_{16:1}\)) extracted by hexane.

References


