MJoCE/Vol 15 No 1 January 2025/Hal. 1-11

p-ISSN 2722-8258
e-ISSN 2722-8266

PRELIMINARY STUDY OF LIPID CONTENT IN BROWN ALGAE (*Sargassum binderi*) FROM MANOKWARI WATERS

Jamius Bin Stepanus

Faculty of Engineering, Papua University, Jl. Gunung Salju Amban, Manokwari, West Papua 98314, Indonesia

jamiusstepanus22@gmail.com

Received: 5 August 2024 / Accepted: 6 September 2024 / Published: 3 January 2025

ABSTRACT

Algae are marine plants with potential as raw materials for biofuel production. The high lipid content in algae can be converted into biofuel through chemical reactions. Research on the chemical composition of algae species found in the West Papua region remains limited. One type of algae commonly found in the waters of West Papua, particularly along the northern coast of Manokwari, is *Sargassum binderi*. Therefore, the aim of this study is to identify the lipid compounds and their percentages in *S. binderi*. Lipid compounds were extracted using two extraction methods: maceration and the Bligh and Dyer method. The extraction process utilized fresh *S. binderi* samples without drying, grinding, or heating treatments. The lipid compounds and their percentages were determined using GC-MS analysis.The study identified six lipid compounds from the maceration method: myristic acid (1.4%), palmitic acid (19.15%), stearic acid (2.71%), oleic acid (5.93%), isooleic acid (4.69%), and arachidonic acid (1.01%), with a total lipid compound percentage of 34.89%. Meanwhile, the Bligh Dyer method identified five lipid compounds: palmitic acid (46.07%), palmitoleic acid (5.23%), oleic acid (4.33%), vaccenic acid (27.18%), and arachidonic acid (2.81%), with a relatively high total lipid compound percentage of 85.62%.

Keywords: *Bligh Dyer, GC-MS, lipid, maceration, Sargassum binderi, wet algae*

INTRODUCTION

The identification of the potential of Papua's local resources can be based on Indonesia's characteristics as a tropical archipelagic country with abundant biodiversity and marine resources. If the focus of studies and research is oriented toward the utilization of marine resources, Indonesia's vast waters can support these objectives.

According to data from *The World Factbook* by the Central Intelligence Agency (CIA) of the United States, Indonesia's coastline is approximately 54,716 km long. Indonesia ranks as the country with the second-longest coastline after Canada among 198 countries and 55 territories worldwide (BPS, 2016). The total area of Indonesia's waters consists of an Exclusive Economic Zone (EEZ) of 2.7 million km^2, an archipelagic sea area of 2.3 million km^2, and territorial waters of 0.8 million km^2 (Likadja, 1985).

West Papua Province, located at the easternmost part of Indonesia, has a sea area of 106,598.9 km^2, with a coastline length of 12,455 km and 3,146 small islands have been identified., according to data from the Geospatial Information Agency in 2018. This data indicates that West Papua has significant economic and strategic potential in its marine resources, which can be utilized for diversifying products, including non-fish products with commercial added value.

One of the most promising marine resources for development is algae. Algae have the potential to be used as biofuel (biodiesel, bioethanol, and biogas) due to their high lipid content (Hannon et al., 2010; Schlagermann et al., 2012; Kumar et al., 2020; Mahmood et al., 2023). These lipid compounds can be converted into biodiesel through specific chemical reactions (Karmee et al., 2015; Talebian-Kiakalaieh & Aishah Saidina, 2019). Biodiesel can serve as an alternative energy source to replace fossil-based diesel fuel.

The use of diesel as a fuel source has several drawbacks, including being a fossil fuel that contributes to air pollution and various health issues. As a fossil fuel, the combustion of diesel

p-ISSN 2722-8258
e-ISSN 2722-8266

releases a significant amount of carbon dioxide into the atmosphere, thereby contributing to global warming (Perera, 2018; Lelieveld et al., 2019; Speight, 2019; Karnauskas et al.,2020). Therefore, research on the characteristics, production, and benefits of biodiesel needs to be developed to address energy security issues, reduce the use of fossil fuels, and promote the nation's economy.

In order to encourage the development of biodiesel in Indonesia, starting in January 2020, the government set a target for the implementation of the Mandatory Biodiesel Program 30 (B30), which requires a 30% biodiesel blend with 70% diesel fuel. This regulation is outlined in the Minister of Energy and Mineral Resources (ESDM) Regulation Number 12 of 2015, which is the third amendment to Minister of ESDM Regulation Number 32 of 2008 regarding the Provision, Utilization, and Trade of Biofuels as Other Fuels.

Therefore, to achieve the B30 Program target and reduce diesel fuel consumption, which has negative impacts, national biodiesel production needs to be increased. So far, in the West Papua region, research related to the utilization of algae for biodiesel production as an alternative to diesel fuel remains very limited. Even literature and studies on the identification of algae species and their distribution are still scarce. Based on these factors, supported by the data presented above, it is necessary to explore the potential of biodiesel from aquatic algae in West Papua as a renewable energy alternative.

One of the algae species commonly found along the coastal areas of West Papua is *Sargassum binderi*. The abundance of this species is the focus of this study. Research on the lipid content of this algae species and its potential as a raw material for biodiesel production remains limited. Therefore, the objectives of this preliminary study are to (1) identify the lipid compounds present in *S. binderi*, (2) determine the percentage of each lipid compound through GC-MS analysis, and (3) determine the extract yield percentage. Lipid extraction was carried out using two simple extraction methods: maceration and the Bligh Dyer method (Garcia-Vaquero et al., 2020; Saini et al., 2021).

RESEARCH METHOD

Sample Preparation

S. binderi algae sample (**Figure 1.(a)**) was obtained from the North Coast of Manokwari, West Papua Province, in mid-July with sampling coordinates of 0°44'45" S, 133°59' E. Before the extraction process, the sample was first rinsed with aquadest to remove any dirt adhering to the surface. Since the extraction process used wet samples, the drying (ElGamal et al., 2023)

(a) (b) (c)

Figure 1. (a) *S. binderi* Algae (b) Maceration Process (c) Bligh-Dyer Process

and grinding stages were not performed. The entire sample was used for extraction, with no part being separated. The sample was cut into small pieces, each less than 0.5 cm in size, and was ready for the extraction process.

Maceration Method

A 50 g sample of *S. binderi* (from the preparation step), was soaked in 500 mL of a analytical grade solvent mixture of *n*-hexane-ethanol (3.5:1.5, v/v) for 3 x 24 hours at room temperature in

p-ISSN 2722-8258
e-ISSN 2722-8266

a dark place. During the maceration process, stirring was carried out using a magnetic stirrer without heating for ±10 minutes every 12 hours. The macerate was then filtered using filter paper to separate the residue. Due to the polarity difference between *n*-hexane and ethanol, two layers were observed: the *n*-hexane macerate in the top layer and the ethanol macerate in the bottom layer (**Figure 1.(b)**). Both macerate layers were separated using a separatory funnel and subsequently evaporated using a rotary evaporator (temperature < 40°C). The macerates were then stored in a freezer (temperature ± 5°C) until futher use.

Bligh Dyer Method

A 50 g sample of *S. binderi* (from the preparation step) was soaked in 300 mL of a analytical grade solvent mixture of methanol-chloroform (2:1, v/v) and stirred with a magnetic stirrer for 1 hour without heating (Saini et al., 2021). Subsequently, 100 mL of chloroform and 100 mL of aquadest were sequentially added, with stirring performed in the same manner and for the same duration after each addition. The extract was then transferred into a separatory funnel and left for 24 hours, resulting in the formation of two layers (**Figure 1.(c)**). The bottom layer which is the chloroform layer is then separated and the chloroform solvent is evaporated using a rotary evaporator (temperature < 40^{o}C) to obtain Bligh Dyer extract. The extract was then stored in a freezer (temperature ± 5^{o}C) until futher use.

Gas Chromatography-Mass Spectrometry Analysis

Lipid compounds were analyzed using a GC-MS instrument (QP2010S Shimadzu) equipped with an Agilent DB-5MS UI column (0.25 mm diameter, 0.25 µm film thickness, and 30 m length). The initial GC-MS temperature was set to 70°C, and the injection temperature was 300°C, with a column flow rate of 0.50 mL/min. The carrier gas (helium) was maintained at a pressure of 13.7 kPa, with a flow rate of 3 mL/min and a linear velocity of 25.9 cm/sec. Lipid compound identification was based on mass spectral comparisons and retention index data of components found in the GC-MS spectral database.

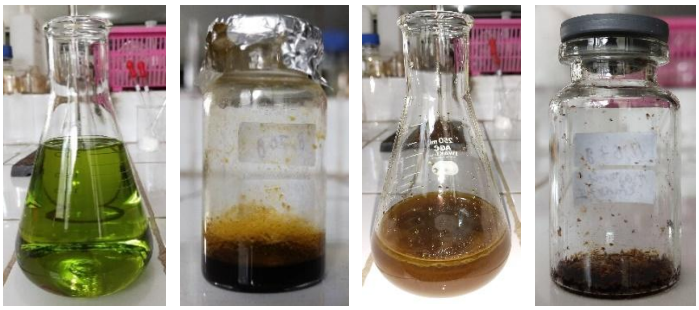
RESULTS AND DISCUSSION

Extraction Results with Maceration Method

Figure 2 (a), (b), (c), and (d) show the extraction results obtained from the maceration method. After evaporation, the *n*-hexane macerate layer which was initially pale green turned into a brownish green precipitate while the ethanol macerate layer became a brown precipitate. The *n*-hexane molecule is nonpolar, whereas the ethanol molecule is polar, leading to the formation of two separate layers in the macerate. Ethanol has a density of 0.7849 g/mL, which is higher than the density of *n*-hexane at 0.6548 g/mL (at 25°C)(Moldoveanu & David, 2015), causing ethanol to settle in the lower layer of the macerate.

N-hexane, ethanol, and chloroform are considered efficient solvents for extracting lipid compounds (Ramluckan et al., 2014). In general, lipid compounds have long alkyl chains (-CH_2-), making them predominantly nonpolar (Small, 1984; Fahy et al., 2005; Vanni et al., 2019). A previous study by Stepanus & Kolibongso (2024) reported that *S. binderi* has a relatively high water content of 85%. Therefore, in this study, a mixture of *n*-hexane and ethanol was used as the extraction solvent.

p-ISSN 2722-8258
e-ISSN 2722-8266



(a) (b) (c) (d)

Figure 2. (a) *n*-hexane Macerate (b) *n*-hexane Macerate after Evaporation (c) Ethanol Macerate (d) Ethanol Macerate after Evaporation

The *n*-hexane molecule (molecular formula C_6H_{14}) is a straight-chain alkane hydrocarbon consisting of six carbon atoms. Each carbon atom in *n*-hexane undergoes sp^3 hybridization, forming only single bonds (inert toward the compounds being extracted) with a tetrahedral bond angle. This results in a non-rigid, non-linear molecular structure. The *n*-hexane molecular chain is nonpolar, allowing it to extract the alkyl chains of lipid compounds through dipole-dipole interactions. The ethanol molecule (molecular formula CH_3CH_2OH), with a single hydroxyl (-OH) functional group, can interact with hydrogen atoms in water molecules (molecular formula H_2O) through hydrogen bonding. Therefore, another function of ethanol as a solvent is to separate water from lipids.

Table 1. Extract from Maceration Method

	***n*-hexane macerate**	**Ethanol macerate**
Extract color	Brownish green	Brown
Extract weight (g)	0.54	0.24
Yield, dry basis (%)	7.2	3.2

Table 1 presents the extraction results from the maceration method. The extract weight from *n*-hexane and ethanol macerates is 0.54 g and 0.24 g, respectively, with a total extract weight of 0.78 g. The extract weight from the *n*-hexane macerate is more than twice that of the ethanol macerate, suggesting that *S. binderi* algae contain relatively more non-polar compounds compared to polar compounds. The percentage yield of the extract (dry basis, %) is calculated based on the initial sample weight (50 g) and the water content in *S. binderi*, which is 85%, which refers to previous research. As a result, the extraction yield percentage from maceration is 10.4%. The observed green-orange-brown coloration in the macerate is attributed to the presence of fucoxanthin and chlorophyll pigments in *S. binderi* (Yip et al., 2014).

GC-MS Analysis Results (Maceration Method)

Figure 3 shows the GC-MS chromatogram of the *S. binderi* extract obtained through maceration. The macerate analyzed using GC-MS is a homogeneous mixture of *n*-hexane and ethanol macerates. The chromatogram reveals a total of 26 peaks, of which 7 peaks represent lipid compounds. As listed in **Table 2**, these seven peaks correspond to peaks 8, 14, 15, 17, 18, 19, and 21. Peaks 14 and 18 correspond to the same lipid compound, 10-octadecenoic acid. Peak 15, representing hexadecanoic acid (palmitic acid), exhibits the highest percentage at 19.15%, followed by 9-octadecenoic acid (Z) (oleic acid) at 5.93% in peak 17.

p-ISSN 2722-8258
e-ISSN 2722-8266

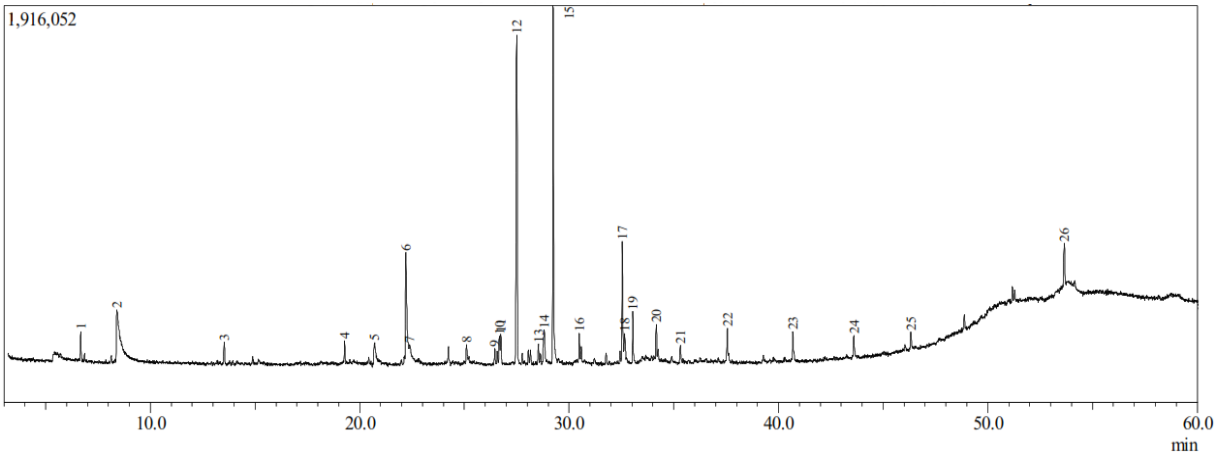


Figure 3. GC-MS chromatogram of *S. binderi* extract (Maceration method)

Table 2. Lipid Compounds Identified by GC-MS from *S. binderi* Extract (Maceration Method)

Peak#	Retention Time	Area	Area%	Similarity	Molecular Formula	Compound
8	25.106	329035	1.4	94	$C_{15}H_{30}O_2$	Tetradecanoic acid, methyl ester
14	28.814	685037	2.91	86	$C_{19}H_{36}O_2$	10-Octadecenoic acid, methyl ester
15	29.237	4514120	19.15	96	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
17	32.544	1397788	5.93	96	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl este
18	32.656	419921	1.78	91	$C_{19}H_{36}O_2$	10-Octadecenoic acid, methyl ester
19	33.044	638267	2.71	95	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester
21	35.315	237536	1.01	88	$C_{21}H_{34}O_2$	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)

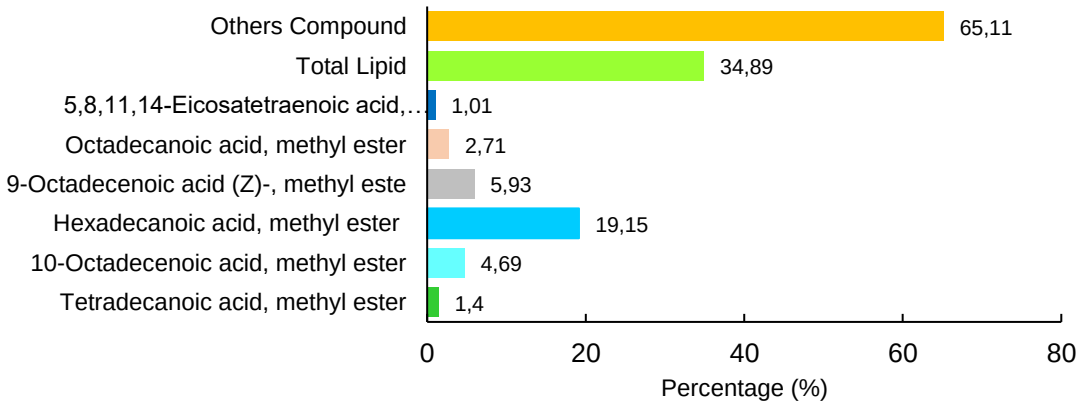


Figure 4. Percentage of Lipid and Non-lipid Compounds (Maceration Method)

Based on the GC-MS analysis results, the percentage graph of lipid and non-lipid compounds from maceration is presented in **Figure 4**. The graph shows that the total percentage of lipid compounds is 34.89%, which is lower than the percentage of non-lipid compounds at 65.11%. Based on this total lipid percentage, the lipid yield obtained from maceration is 0.27 g (from the total macerate of 0.78 g) or 0.54% (from the initial sample weight of 50 g).

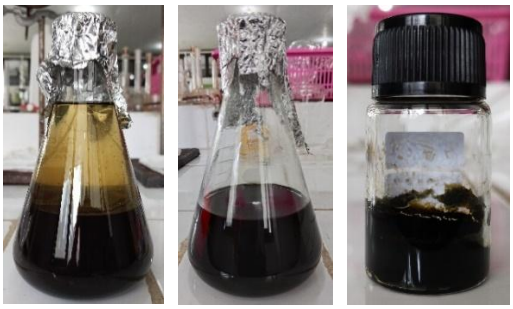
The lipid compounds obtained from maceration were converted into fatty acid methyl esters (FAME) through the esterification/transesterification process. Subsequently, the types of fatty

p-ISSN 2722-8258
e-ISSN 2722-8266

acids were identified. Based on the analysis, the identified fatty acids include myristic acid (tetradecanoic acid, C14:0), palmitic acid (hexadecanoic acid, C16:0), stearic acid (octadecanoic acid, C18:0), oleic acid (9-octadecenoic acid (Z), C18:1, cis-9), isooleic acid (10-octadecenoic acid, C18:1), and arachidonic acid (5,8,11,14-eicosatetraenoic acid, C20:4, ω-6). These types of fatty acids are suitable raw materials for biodiesel production (Rattanaphra et al., 2011; Moradi et al., 2021; Sánchez-Cupil et al., 2024).

Extraction Results with Bligh Dyer Method

The extraction results using the Bligh-Dyer method are shown in **Figure 5** (a), (b), and (c). The upper layer observed in **Figure 5**(a) corresponds to the aquadest-methanol layer, while the lower layer is the chloroform layer. The difference in polarity between aquadest-methanol and chloroform results in the formation of two distinct layers. The molecules of aquadest (distilled water, H_2O) and methanol (CH_3OH) are polar, whereas chloroform ($CHCl_3$) is semipolar. The densities of aquadest, methanol, and chloroform are 0.9970 g/mL, 0.7866 g/mL, and 1.4798 g/mL, respectively, at 25°C (Moldoveanu & David, 2015), which causes the aquadest-methanol



(a) (b) (c)

Figure 5. (a) Two Layers of Bligh-Dyer Extract (b) Chloroform layer (c) Chloroform Layer after Evaporation

layer to remain on top. The evaporation of the chloroform layer yielded a dark green precipitate. During the evaporation process using a rotary evaporator, both in maceration and Bligh Dyer extraction, the temperature was maintained below 40°C to prevent the degradation of compounds due to high-temperature heating (Wang et al., 2018; Bennour et al., 2020).

Table 3. Extract from Bligh Dyer Method

Extract color	Dark green
Extract weight (g)	0.27
Yield, dry basis (%)	3.6

Table 3 presents the extraction results using the Bligh Dyer method. The extract weight from the chloroform layer was 0.27 g (almost three times lower compared to the maceration extract, which was 0.78 g). The extraction yield obtained was 3.6%. In the Bligh Dyer method, lipid compounds are extracted into the chloroform layer, while water and other polar compounds are extracted into the aqueous-methanol layer. Chloroform molecules consist of a methane molecule substituted with three Cl atoms. Since chlorine atoms are more electronegative than carbon and hydrogen atoms, the chloroform molecule is semi-polar, unlike methane, which is non-polar. Consequently, lipids, which are generally non-polar, are extracted into the chloroform layer.

The molecules CH_3Cl, CH_3OH, and H_2O have relatively small molecular structures, facilitating diffusion in and out of algal cells during extraction. However, due to their small molecular size, their ability to interact with and extract larger molecules decreases. This is suspected to be the reason for the lower extract weight obtained using the Bligh Dyer method.

p-ISSN 2722-8258
e-ISSN 2722-8266

GC-MS Analysis Results (Bligh Dyer Method)

Unlike the chromatogram from maceration, the chromatogram from the Bligh Dyer method shows a total of only nine peaks, as seen in **Figure 6**. Although there are only nine peaks, five of them represent fatty acid compounds. These five peaks correspond to peaks 1, 2, 3, 4, and 6. The identified fatty acids include palmitic acid (hexadecanoic acid, C16:0), palmitolinoleic acid (9,12-hexadecadienoic acid, C16:2), oleic acid (9-octadecenoic acid (Z), C18:1, cis-9), vaccenic acid (11-octadecenoic acid, C18:1), and arachidonic acid (5,8,11,14-eicosatetraenoic acid, C20:4, ω-6).

Similar to the maceration results, the Bligh Dyer method also recorded palmitic acid as having the highest percentage, at 46.07%, nearly half of the total extract, which can be seen at peak 2. In addition to palmitic acid, the peaks for oleic acid and arachidonic acid were also observed in the maceration chromatogram. However, the peaks for palmitolinoleic acid and vaccenic acid were not detected in the maceration chromatogram. The vaccenic acid compound at peak 4 has the second-highest percentage at 27.18%.

Palmitic acid, $CH_3(CH_2)_{14}COOH$, has a relatively smaller molecular structure compared to other identified fatty acid compounds. This small particle/molecular size facilitates the diffusion process out of the sample cell wall during extraction (Zhang et al., 2018; Al Ubeed et al., 2022), resulting in the highest recorded percentage. Noviendri et al. (2011) also reported that palmitic acid is the dominant lipid compound extracted from the same sample. Conversely, arachidonic acid, which has the largest structural size, exhibits the lowest percentage.

Based on the type and percentage of fatty acids identified from both extraction methods, it can be stated that the algae *S. binderi* contains lipids with potential for biodiesel production. In addition to *S. binderi*, several previous studies have reported the potential of other brown algae species as sources of biogas, bioethanol, and biodiesel (Orozco-González et al., 2022). These include *Sargassum angustifolium* (Ardalan et al., 2018), *Sargassum angustifolium*, *Sargassum boveanum*, *Sargassum vulgare* (Jeliani et al., 2021) and *Sargassum latifolium* (El-Gendy et al., 2024).

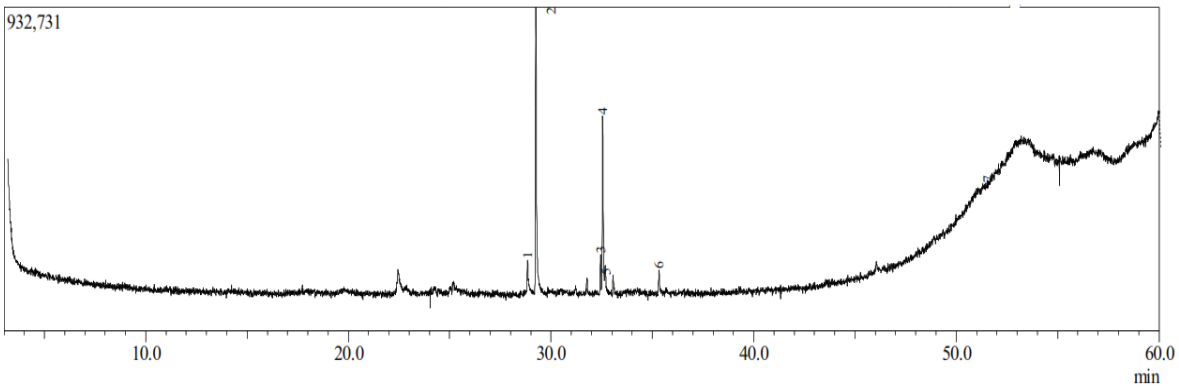


Figure 6. GC-MS Chromatogram of *S. binderi* Extract (Bligh Dyer Method)

Table 4. Lipid Compounds Identified by GC-MS from *S. binderi* Extract (Bligh Dyer Method)

Peak	Retention Time	Area	Area%	Similarity	Molecular Formula	Compound
1	28.842	165121	4.33	91	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl ester
2	29.251	1756967	46.07	97	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
3	32.442	199364	5.23	93	$C_{17}H_{30}O_2$	9,12-Hexadecadienoic acid, methyl ester
4	32.548	1036506	27.18	95	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester, (Z)-
6	35.33	107079	2.81	91	$C_{21}H_{34}O_2$	5,8,11,14-Eicosatetraenoic acid, methyl ester (all-Z)-

p-ISSN 2722-8258
e-ISSN 2722-8266

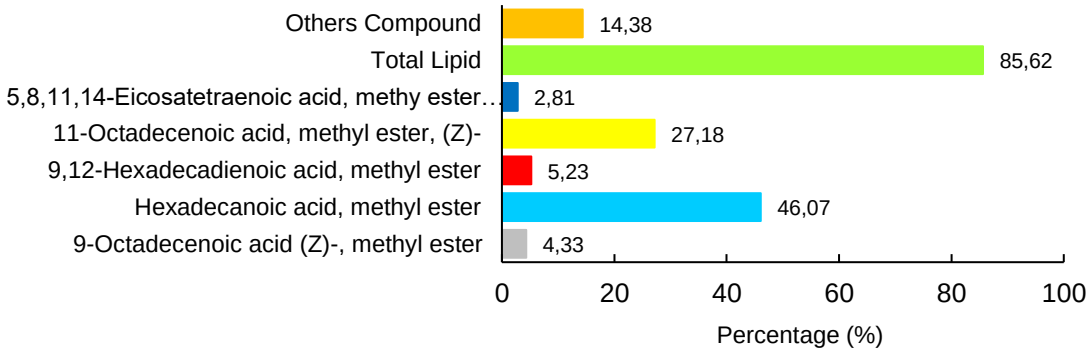


Figure 7. Percentage of Lipid and Non-lipid Compounds (Bligh Dyer method)

Based on the graph in **Figure 7**, the total percentage of detected lipid compounds is relatively high, at 85.62%. de Jesus et al. (2019) reported that the Bligh and Dyer method yields a high lipid recovery from wet microalgae extracts. In this study, GC-MS analysis identified six lipid compounds using the maceration method, whereas the Bligh Dyer method identified five lipid compounds. Meanwhile, a previous study using the Soxhlet method identified only four lipid compounds. Among these four compounds, palmitic acid had a relatively high percentage of 18.12%, with a small difference compared to oleic acid, which had the highest percentage at 19.4% (Stepanus & Kolibongso, 2024).

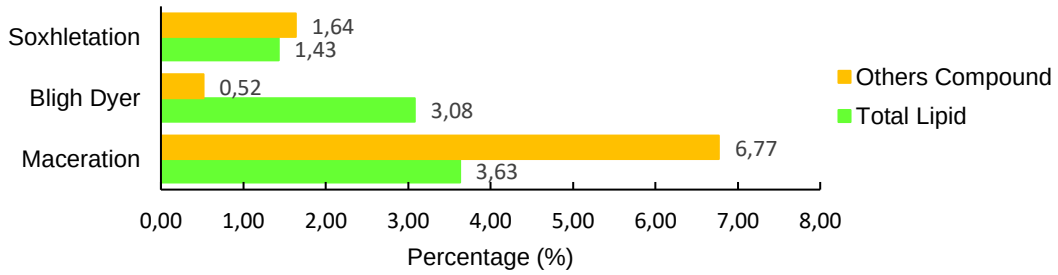


Figure 8. Comparison of Yield Percentages (Dry Basis, %) from Three Extraction Methods

Previous studies have reported that different extraction methods (such as preparation treatments, solvent type, extraction time, temperature, and other factors) yield varying lipid percentages depending on the species of algae/microalgae being studied (Prommuak et al., 2012; Soares et al., 2014; Hussain et al., 2015; Hurtado et al., 2018). **Figure 8** presents the total yield percentages for lipid and non-lipid compounds extracted using the two extraction methods in this study, compared to the Soxhlet method. The yield percentage calculations are based on dry weight (dry basis). According to the graph, the lipid yield percentages from the maceration and Bligh and Dyer methods are similar, at around 3%, whereas the yield from the Soxhlet method is lower, at 1.43%.

CONCLUSION

Based on the research results, six lipid compounds were identified using the maceration method, with a total percentage of 34.89%. Meanwhile, five lipid compounds were identified using the Bligh Dyer method, with a total percentage of 85.62%.The lipids extracted using the maceration method consisted of myristic acid (1.4%), palmitic acid (19.15%), stearic acid (2.71%), oleic acid (5.93%), isooleic acid (4.69%), and arachidonic acid (1.01%). Meanwhile, the lipids extracted using the Bligh Dyer method consisted of palmitic acid (46.07%), palmitoleic acid

p-ISSN 2722-8258
e-ISSN 2722-8266

(5.23%), oleic acid (4.33%), vaccenic acid (27.18%), and arachidonic acid (2.81%). The lipid yield percentages from the maceration and Bligh Dyer methods were 3.08% and 3.63%, respectively.

REFERENCES

Al Ubeed, H. M. S., Bhuyan, D. J., Alsherbiny, M. A., Basu, A., & Vuong, Q. V. (2022). A Comprehensive Review on the Techniques for Extraction of Bioactive Compounds from Medicinal Cannabis. *Molecules*, *27*(3), 1–18. https://doi.org/10.3390/molecules27030604

Ardalan, Y., Jazini, M., & Karimi, K. (2018). Sargassum angustifolium brown macroalga as a high potential substrate for alginate and ethanol production with minimal nutrient requirement. *Algal Research*, *36*(October), 29–36. https://doi.org/10.1016/j.algal.2018.10.010

Bennour, N., Mighri, H., Eljani, H., Zammouri, T., & Akrout, A. (2020). Effect of solvent evaporation method on phenolic compounds and the antioxidant activity of Moringa oleifera cultivated in Southern Tunisia. *South African Journal of Botany*, *129*, 181–190. https://doi.org/10.1016/j.sajb.2019.05.005

BPS. (2016). *Statistik Sumber Daya Laut dan Pesisir (Statistics of Marine and Coastal Resources) 2016* (S. D. of E. Statistics (ed.)). BPS-Statistics Indonesia.

de Jesus, S. S., Ferreira, G. F., Moreira, L. S., Wolf Maciel, M. R., & Maciel Filho, R. (2019). Comparison of several methods for effective lipid extraction from wet microalgae using green solvents. *Renewable Energy*, *143*, 130–141. https://doi.org/10.1016/j.renene.2019.04.168

El-Gendy, N. S., Hosny, M., Ismail, A. R., Radwan, A. A., Ali, B. A., Ali, H. A., El-Salamony, R. A., Abdelsalam, K. M., & Mubarak, M. (2024). A Study on the Potential of Valorizing Sargassum latifolium into Biofuels and Sustainable Value-Added Products . *International Journal of Biomaterials*, *2024*(1). https://doi.org/10.1155/2024/5184399

ElGamal, R., Song, C., Rayan, A. M., Liu, C., Al-Rejaie, S., & ElMasry, G. (2023). Thermal Degradation of Bioactive Compounds during Drying Process of Horticultural and Agronomic Products: A Comprehensive Overview. *Agronomy*, *13*(6). https://doi.org/10.3390/agronomy13061580

Fahy, E., Subramaniam, S., Brown, H. A., Glass, C. K., Merrill, A. H., Murphy, R. C., Raetz, C. R. H., Russell, D. W., Seyama, Y., Shaw, W., Shimizu, T., Spener, F., Van Meer, G., VanNieuwenhze, M. S., White, S. H., Witztum, J. L., & Dennis, E. A. (2005). A comprehensive classification system for lipids. *Journal of Lipid Research*, *46*(5), 839–861. https://doi.org/10.1194/jlr.E400004-JLR200

Garcia-Vaquero, M., Rajauria, G., & Tiwari, B. (2020). Conventional extraction techniques: Solvent extraction. In *Sustainable Seaweed Technologies: Cultivation, Biorefinery, and Applications*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-817943-7.00006-8

Hannon, M., Gimpel, J., Tran, M., Rasala, B., & Mayfield, S. (2010). Biofuels from algae: challenges and potential Importance & challenges of algal biofuels. *Biofuels*, *1*(5), 763–784. http://www.future-science.com/doi/abs/10.4155/bfs.10.44%0Ahttps://www.ncbi.nlm.nih.gov/pmc/articles/PMC3152439/pdf/nihms269384.pdf

Hurtado, D. X., Garzón-Castro, C. L., Cortés-Romero, J., & Camacho, E. T. (2018). Comparison of lipid extraction methods for the microalgae Acutodesmus obliquus. *International Journal of Agricultural and Biological Engineering*, *11*(5), 211–217. https://doi.org/10.25165/j.ijabe.20181105.3748

Hussain, J., Liu, Y., Lopes, W. A., Druzian, J. I., Souza, C. O., Carvalho, G. C., Nascimento, I. A., & Liao, W. (2015). Effects of Different Biomass Drying and Lipid Extraction Methods on Algal Lipid Yield, Fatty Acid Profile, and Biodiesel Quality. *Applied Biochemistry and Biotechnology*, *175*(6), 3048–3057. https://doi.org/10.1007/s12010-015-1486-5

Jeliani, Z. Z., Fazelian, N., & Yousefzadi, M. (2021). Introduction of macroalgae as a source of.pdf. *Journal of the Marine Biological Association of the United Kingdom 1–8.*, *101*(3), 527–534.

p-ISSN 2722-8258
e-ISSN 2722-8266

Karmee, S. K., Linardi, D., Lee, J., & Lin, C. S. K. (2015). Conversion of lipid from food waste to biodiesel. *Waste Management*, *41*, 169–173. https://doi.org/10.1016/j.wasman.2015.03.025

Karnauskas, K. B., Miller, S. L., & Schapiro, A. C. (2020). Fossil Fuel Combustion Is Driving Indoor CO2 Toward Levels Harmful to Human Cognition. *GeoHealth*, *4*(5), 0–1. https://doi.org/10.1029/2019GH000237

Kumar, M., Sun, Y., Rathour, R., Pandey, A., Thakur, I. S., & Tsang, D. C. W. (2020). Algae as potential feedstock for the production of biofuels and value-added products: Opportunities and challenges. *Science of the Total Environment*, *716*, 137116. https://doi.org/10.1016/j.scitotenv.2020.137116

Lelieveld, J., Klingmüller, K., Pozzer, A., Burnett, R. T., Haines, A., & Ramanathan, V. (2019). Effects of fossil fuel and total anthropogenic emission removal on public health and climate. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(15), 7192–7197. https://doi.org/10.1073/pnas.1819989116

Likadja, F. E. (1985). Hukum Laut dan Undang-Undang Perikanan. *Seminar Pengembangan Dan Pemanfaatan Sumber Daya Akuatik Antara Perguruan Tinggi Dan BAPPEDA Se-Indonesia Bagian Timur*. https://jurnal.hukumonline.com/a/5cb49ddc01fb73001038d09f/hukum-laut-dan-undang-undang-perikanan/

Mahmood, T., Hussain, N., Shahbaz, A., Mulla, S. I., Iqbal, H. M. N., & Bilal, M. (2023). Sustainable production of biofuels from the algae-derived biomass. *Bioprocess and Biosystems Engineering*, *46*(8), 1077–1097. https://doi.org/10.1007/s00449-022-02796-8

Moldoveanu, S., & David, V. (2015). Solvent Extraction. In *Modern Sample Preparation for Chromatography* (pp. 131–189). https://doi.org/10.1016/b978-0-444-54319-6.00006-2

Moradi, P., Saidi, M., & Najafabadi, A. T. (2021). Biodiesel production via esterification of oleic acid as a representative of free fatty acid using electrolysis technique as a novel approach: Non-catalytic and catalytic conversion. *Process Safety and Environmental Protection*, *147*, 684–692. https://doi.org/10.1016/j.psep.2020.12.032

Noviendri, D., Jaswir, I., Salleh, H. M., Taher, M., Miyashita, K., & Ramli, N. (2011). Fucoxanthin extraction and fatty acid analysis of Sargassum binderi and S. duplicatum. *Journal of Medicinal Plants Research*, *5*(11), 2405–2412.

Orozco-González, J. G., Amador-Castro, F., Gordillo-Sierra, A. R., García-Cayuela, T., Alper, H. S., & Carrillo-Nieves, D. (2022). Opportunities Surrounding the Use of Sargassum Biomass as Precursor of Biogas, Bioethanol, and Biodiesel Production. *Frontiers in Marine Science*, *8*(January), 1–11. https://doi.org/10.3389/fmars.2021.791054

Perera, F. (2018). Pollution from fossil-fuel combustion is the leading environmental threat to global pediatric health and equity: Solutions exist. *International Journal of Environmental Research and Public Health*, *15*(1). https://doi.org/10.3390/ijerph15010016

Prommuak, C., Pavasant, P., Quitain, A. T., Goto, M., & Shotipruk, A. (2012). Microalgal lipid extraction and evaluation of single-step biodiesel production. *Engineering Journal*, *16*(5), 157–166. https://doi.org/10.4186/ej.2012.16.5.157

Ramluckan, K., Moodley, K. G., & Bux, F. (2014). An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the soxhlet extraction method. *Fuel*, *116*(September), 103–108. https://doi.org/10.1016/j.fuel.2013.07.118

Rattanaphra, D., Harvey, A. P., Thanapimmetha, A., & Srinophakun, P. (2011). Kinetic of myristic acid esterification with methanol in the presence of triglycerides over sulfated zirconia. *Renewable Energy*, *36*(10), 2679–2686. https://doi.org/10.1016/j.renene.2011.02.018

Saini, R. K., Prasad, P., Shang, X., & Keum, Y. S. (2021). Advances in lipid extraction methods—a review. *International Journal of Molecular Sciences*, *22*(24), 1–19. https://doi.org/10.3390/ijms222413643

Sánchez-Cupil, J. L., Cuevas-García, R., Ramírez, J., Gutiérrez-Alejandre, A., & Jiménez-Díaz, M. L. (2024). Green diesel production using stearic and palmitic acids on Ni catalysts obtained from Ternary Hydrotalcites Ni-Mg–Al. *Biomass Conversion and Biorefinery*, *14*(18), 22073–22086. https://doi.org/10.1007/s13399-023-04417-0

p-ISSN 2722-8258
e-ISSN 2722-8266

Schlagermann, P., Göttlicher, G., Dillschneider, R., Rosello-Sastre, R., & Posten, C. (2012). Composition of algal oil and its potential as biofuel. *Journal of Combustion*, *2012*. https://doi.org/10.1155/2012/285185

Small, D. M. (1984). Lateral chain packing in lipids and membranes. *Journal of Lipid Research*, *25*(13), 1490–1500. https://doi.org/10.1016/s0022-2275(20)34422-9

Soares, A. T., da Costa, D. C., Silva, B. F., Lopes, R. G., Derner, R. B., & Antoniosi Filho, N. R. (2014). Comparative Analysis of the Fatty Acid Composition of Microalgae Obtained by Different Oil Extraction Methods and Direct Biomass Transesterification. *Bioenergy Research*, *7*(3), 1035–1044. https://doi.org/10.1007/s12155-014-9446-4

Speight, J. G. (2019). Energy security and the environment. *Natural Gas*, 361–390. https://doi.org/10.1016/b978-0-12-809570-6.00010-2

Stepanus, J. Bin, & Kolibongso, D. (2024). Exploration of Lipid Content in Sargassum binderi Algae from the North Coast of Manokwari West Papua. *Indonesian Journal of Chemical Science and Technology*, *07*(1), 43–50. https://doi.org/10.24114/ijcst.v7i1.56442

Talebian-Kiakalaieh, A., & Aishah Saidina, A. N. (2019). Conversion of lipids to biodiesel via esterification and transesterification. *Chemical Catalysts for Biomass Upgrading*, 439–468. https://doi.org/10.1002/9783527814794.ch10

Vanni, S., Riccardi, L., Palermo, G., & De Vivo, M. (2019). Structure and Dynamics of the Acyl Chains in the Membrane Trafficking and Enzymatic Processing of Lipids. *Accounts of Chemical Research*, *52*(11), 3087–3096. https://doi.org/10.1021/acs.accounts.9b00134

Wang, L., Liu, Y., Pu, F., Zhang, W., & Zhou, Z. (2018). Effect of Rotary Evaporator Water Bath Temperature on Recovery Rate of Phthalate Esters. *Bulletin of Environmental Contamination and Toxicology*, *101*(6), 810–813. https://doi.org/10.1007/s00128-018-2440-3

Yip, W. H., Lim, S. J., Mustapha, W. A. W., Maskat, M. Y., & Said, M. (2014). Characterisation and stability of pigments extracted from Sargassum binderi obtained from Semporna, Sabah. *Sains Malaysiana*, *43*(9), 1345–1354.

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine (United Kingdom)*, *13*(1), 1–26. https://doi.org/10.1186/s13020-018-0177-x