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Microwave-Assisted Deep Eutectic Solvent Extraction of Lipids from *Ulva sp.*: Optimization and Fatty Acid Profiling

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Abstract: *Ulva sp.* is a green alga commonly found in Indonesian waters. It contains approximately 60% carbohydrates, 10–47% protein, and 1–3% lipids. This study aimed to determine the optimum conditions for lipid extraction from *Ulva sp.* A pre-treatment step was carried out by adding a Deep Eutectic Solvent (DES) and distilled water. The DES used was a mixture of zinc chloride and urea in a 1:2 molar ratio. The sample was then mixed with a chloroform-methanol solvent (2:1 v/v) and subjected to Microwave-Assisted Extraction (MAE). The variables investigated in this study included microwave power (150, 300, and 450 W), extraction time (10, 15, and 20 min), and solvent volume (45, 60, and 75 mL). The organic phase obtained from the extraction process was separated and evaporated to determine the lipid yield. The highest yield, 15.8%, was achieved under conditions of 20 min extraction time, 75 mL solvent volume, and 300 W of power. GC-MS analysis of the highest-yield sample revealed the presence of fatty acids including palmitic acid, oleic acid, and palmitoleic acid. This optimized method supports future applications in biodiesel production and green extraction processes for algae-based bioresources.

Keywords: Deep Eutectic Solvent; Extraction; Lipid; Microwave; *Ulva sp.*

Introduction

Ulva sp., a green macroalga [1] [2] commonly found in Indonesian coastal waters, has attracted attention due to its high carbohydrate (≈60%) and moderate protein content (10–47%), despite its relatively low lipid fraction (1–3%) [3]. However, its abundance, rapid growth, and ease of cultivation make it a promising biomass feedstock for lipid-based applications in the cosmetic, food industry, and pharmaceutical [4].

Efficient lipid extraction from algal biomass is a critical step in realizing its potential as a renewable bioresource. The study conducted by Ramola et al. reported that lipid extraction from Oedogonium macroalgae yielded 14% using the Soxhlet extraction method over a 6-hour process with a chloroform-methanol (2:1) solvent system. The use of organic solvents resulted in maximum lipid extraction yield [5]. Meanwhile, the study by Zou et al. obtained a 6% lipid yield from dried Chlorella vulgaris using chloroform-methanol (1:2) with the Bligh and Dyer method [6]. Traditional extraction methods such as Soxhlet and solvent-based techniques are often time-consuming and require large volumes of hazardous solvents. In contrast, Microwave-Assisted Extraction (MAE) offers a faster and more energy-efficient alternative, facilitating cell disruption and enhancing mass transfer. The study by Le et al. (2019) on the extraction of polysaccharides and antioxidants from Ulva pertusa using Microwave-Assisted Extraction (MAE) is one example of research conducted on *Ulva sp.* [7].

Deep Eutectic Solvents (DES) are eutectic mixtures of acids and bases that contain various anionic and cationic entities [8]. DES can also be defined as a mixture of two or more components capable of self-association through hydrogen bonding interactions, resulting in a significant decrease in melting point at a specific composition (eutectic composition) [9]. The melting point of the mixture is lower than that of any individual component. This depression in melting point is attributed to the formation of intramolecular hydrogen bonds, and DES are characterized by low toxicity and recyclability [10]. DES can be categorized into four types: mixtures of metal salts and organic salts, hydrates of metal salts and organic salts, hydrogen bond donors and organic salts, and metal chlorides with hydrogen bond donors. One example of the latter type is a DES composed of urea and $ZnCl_2$ [9]. A study by Weidong et al. [11] demonstrated that the lipid recovery ratio increased significantly when using aDES (a combination of distilled water and DES), achieving up to 80% lipid recovery. Lipid recovery refers to the amount of lipid extracted relative to the total lipid content in the biomass. Prior to aDES treatment, lipid content was only about 15%. The effectiveness of lipid recovery can also be observed through carbohydrate content, which decreased by approximately 20.14%–24.21% after aDES treatment [11]. Recent studies have demonstrated that the addition of DES during pretreatment can further improve lipid yield. DES, particularly those composed of zinc chloride and urea, have shown potential as green solvents due to their low toxicity, biodegradability, and ability to

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disrupt cell wall structures through hydrogen bonding interactions.

Despite its potential, studies on the use of MAE combined with DES for lipid extraction from *Ulva sp.* remain limited. Most existing research has focused on antioxidant or polysaccharide extraction, leaving a gap in the development of optimized lipid extraction strategies for this species. To the best of our knowledge, this is the first study that optimizes lipid extraction from *Ulva sp.* using a synergistic combination of DES pretreatment and MAE, analyzed through Response Surface Methodology (RSM). This approach not only fills a methodological gap but also expands the valorization potential of *Ulva sp.* for bio-based lipid applications.

Therefore, this study aims to optimize lipid extraction from *Ulva sp.* using a combination of DES pretreatment and MAE. The effects of extraction time, solvent volume, and microwave power were investigated using Response Surface Methodology (RSM), and the lipid composition of the best-performing extract was further analyzed by GC-MS.

Research Methods

Raw Material Treatment

A total of 4 kg of *Ulva sp.* algae was collected and washed under running water to remove impurities. The algae were then dried in an oven at 60°C until a moisture content of 19.2% was reached. After drying, the algae were ground using a blender and stored in a dry, dark place to avoid direct sunlight exposure. The purpose of this grinding process was to obtain fine, dry powder with a particle size of 60 mesh, which was then used in the subsequent experimental steps.

Preparation of Deep Eutectic Solvent

The DES was prepared using zinc chloride and urea in a 1:2 molar ratio. The mixture was heated at 80°C until a colorless liquid was formed. The aqueous DES (aDES) was prepared by mixing 3 mL of DES with 20 mL of water to reduce viscosity and enhance mass transfer during extraction. Pretreatment of the algae using aDES was carried out at room temperature for 24 hours with continuous stirring using a magnetic stirrer. A sample of 1 gram of algae was used for the pretreatment process [12].

Microwave-Assisted Extraction

A 1-gram biomass of Ulva pretreated with aDES was mixed with a chloroform and methanol mixture. The chloroform-methanol solvent (2:1) was added to each extraction sample. Extraction was carried out using a Microwave-Assisted Extraction (MAE) device, based on varying extraction time, solvent-to-biomass ratio, and microwave power. The algal residue was first separated by filtration using filter paper. The resulting liquid was further separated using a separatory funnel to isolate the extract (organic phase), which was collected from the lower layer. The extract was then evaporated using a rotary evaporator at 50°C and 50 rpm. The evaporated product was collected, weighed, and stored at 20°C until further analysis. Each experimental condition was conducted in duplicate (n = 2) to ensure reproducibility of the lipid yield results.

The yield (%) was calculated as follows:

$$\text{Yield} = \frac{\text{lipid extract weight (g)}}{\text{weight of sample (g)}} 100\%$$

Yield data analysis was conducted using Design Expert software version 13. The approach applied was Response Surface Methodology (RSM) with a Box-Behnken Design (BBD) consisting of 17 experimental runs, as shown in Table 1.

Lipid Characterization

The extracted samples were transesterified and analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) and Thin Layer Chromatography (TLC). Samples with the highest and lowest yields were selected for GC-MS analysis to determine the content and characteristics of the extracted lipids. GC-MS is a method used to analyze the composition of various compounds in a sample.

Thin Layer Chromatography (TLC) testing was performed to separate and identify compounds in the sample mixture. In this study, TLC was used to detect the presence of fatty acids. The procedure involved spotting the TLC plate with the sample, followed by immersion in a mobile phase composed of hexane-methyl acetate-acetic acid in an 80:20:1 ratio. The height of the mobile phase was carefully maintained below the application line on the TLC plate during development. The TLC plate was immersed in a tightly sealed container, allowed to develop, and then dried at room temperature. Visualization was carried out using UV light at a wavelength of 365 nm by illuminating the developed TLC plate.

Results and Discussion

Yield Results

Lipid extraction from *Ulva sp.* was carried out using the Microwave-Assisted Deep Eutectic Solvent Extraction method. The process variables observed to evaluate their effect on yield included extraction time (10–20 min), solvent volume (45–75 mL), and microwave power (150–450 W). The extraction process involved a pre-treatment step using a DES, composed of zinc chloride and urea in a 1:2 ratio. The selected levels of microwave power (150, 300, and 450 W) and solvent volumes (45, 60, and 75 mL) were determined based on preliminary trials and literature reports that suggested these ranges optimize lipid extraction efficiency without degrading product quality. Extraction was then performed using MAE under the operating conditions listed in Table 1. The lipid yield obtained from each sample is presented in Table 1.

Table 1. Lipid Extraction Yield Results of *Ulva sp.*

Run	Time (min)	Solvent Volume (mL)	Power (W)	*Yield* %
1	15	45	150	5.23
2	15	45	450	12.2
3	10	75	300	9.37
4	15	75	450	15.2
5	20	60	150	2.11
6	15	60	300	9.6
7	20	60	450	10.87
8	15	60	300	10.76
9	15	60	300	10.29

10	15	75	150	12.5
11	15	60	300	11.79
12	10	45	300	4.29
13	10	60	150	3.79
14	20	45	300	2.61
15	20	75	300	15.8
16	15	60	300	10.58
17	10	60	450	7.7

Table 1 shows that the highest lipid yield obtained in this study was 15.8%, achieved by sample 15 under the conditions of 20 min extraction time, 75 mL solvent volume, and 300 W of microwave power. The lowest yield was 2.11%, obtained from sample 5 with 20 min of extraction time, 60 mL of solvent, and 150 W of power. A study by Karuppaswamy et al. (2022) reported a lipid yield of 4.21% from *Ulva lactuca* using Soxhlet extraction and a chloroform–methanol solvent system [13]. These findings indicate that the addition of DES can facilitate cell wall disruption and enhance lipid release. The pre-treatment process with DES promotes lipid extraction by increasing the permeability of the algal cell wall. This is also attributed to the reduction in hydrogen bonding energy within macromolecules (such as cellulose and hemicellulose), as hydrogen bonds are formed between the aDES supermolecules and cell wall macromolecules [11]. The experimental results were further analyzed using ANOVA with Design Expert version 13 to evaluate the effect of extraction variables.

ANOVA Results

Analysis of Variance (ANOVA) is a statistical method used to estimate which variables have the most significant effect based on the relationships among other variables [14]. The ANOVA results in Table 2 show that the extraction variables had a significant influence on the response. The significance of the model is determined by the F-value and p-value, which were 15.96 and 0.0007%, respectively. A high F-test value along with a low p-value indicates that the model's significance has a strong effect on the experimental results [15].

The main criteria used for optimization include model significance ($p < 0.05$) and lack of fit [16]. In ANOVA, a variable is considered significant when $p < 0.05$, and not significant when $p > 0.10$, while the lack of fit must be non-significant. Other parameters include an R^2 value greater than 0.7 and adequate precision greater than 4 [17].

Table 2. Analysis of Variance (ANOVA) for the Response Results

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	266.58	9	29.62	15.96	0.0007	significant
A-Waktu	4.87	1	4.87	2.62	0.1494	
B-Jumlah pelarut	101.82	1	101.82	54.86	0.0001	
C-Daya	62.38	1	62.38	33.61	0.0007	
AB	16.44	1	16.44	8.86	0.0206	
AC	5.88	1	5.88	3.17	0.1183	
BC	4.56	1	4.56	2.46	0.1611	
A^2	63.25	1	63.25	34.08	0.0006	
B^2	7.00	1	7.00	3.77	0.0933	
C^2	1.57	1	1.57	0.8463	0.3882	
Residual	12.99	7	1.86			
Lack of Fit	10.45	3	3.48	5.49	0.0667	not significant
Pure Error	2.54	4	0.6345			
Cor Total	279.57	16				

The analysis results showed that the developed model was significant, with an F-value of 15.96 and a Prob > F of 0.0007 ($p < 0.05$). The lack of fit was not significant, with a value of 0.0667 ($p > 0.05$). A non-significant lack of fit indicates that the model used is appropriate and can adequately explain the observed data [18]. These findings suggest that the model is suitable for studying the effect of process variables on lipid yield.

The time variable had a p-value greater than 0.05, indicating it did not have a significant effect on yield. This is because the highest yield was not obtained at the longest extraction time, nor was the lowest yield obtained at the shortest extraction time [19]. In contrast, the solvent volume and microwave power variables had p-values less than 0.05, indicating a significant effect on yield. Among these, solvent volume was the most significant variable, with the lowest p-value of 0.0001 compared to time and power.

The analysis of variance also produced a linear regression relationship between the predicted values from the model and the experimental data, as shown in the parity plot in Figure 1. The straight line on the parity plot indicates that the model's predicted values closely match the actual experimental results, supported by an R^2 value approaching 100%. This trend confirms that the developed model can reliably predict lipid yield in the experimental setup.

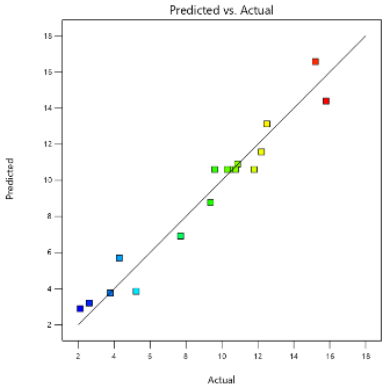


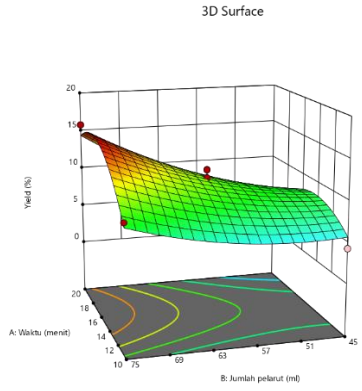
Figure 1. Parity Plot (Predicted vs Actual)

R-Squared (R^2) is the coefficient of determination, which ranges from 0 to 1. If R^2 approaches 1, it indicates a stronger relationship between the variables. Conversely, a lower R^2 value indicates a weaker relationship between the variables [20]. The R^2 value obtained from the analysis in Table 3 is 0.9535. The model for each response is considered to meet the criteria if the R^2 value is greater than 0.7 and the adequate precision value is greater than 4 [21].

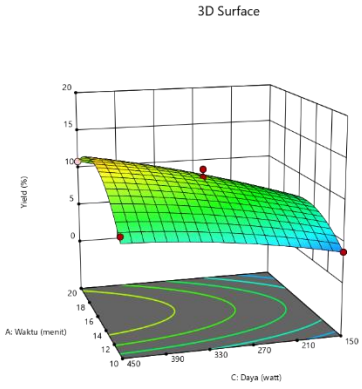
Table 3. ANOVA Fit Statistics

Std. Dev.	1.36	R^2	0.9535
Mean	9.10	Adjusted R^2	0.8938
C.V. %	14.97	Predicted R^2	0.3876
		Adeq Precision	13.0951

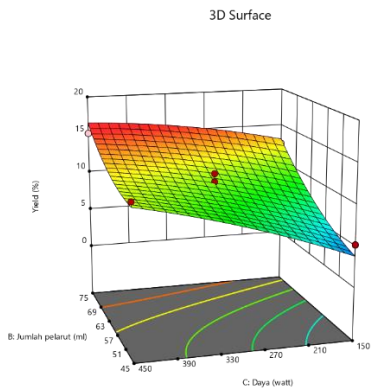
The ANOVA results in Table 3 indicate that the model does not fully meet the required statistical criteria. The adjusted R^2 obtained was 0.8938, while the predicted R^2 was 0.3876. The difference between the adjusted and predicted R^2 values exceeded the acceptable threshold of 0.2, with a gap of 0.5062, suggesting a potential issue with the model or the data used. This discrepancy may arise due to the presence of non-significant independent variables, which limit their ability to explain the response accurately [22]. This limitation may have been influenced by insufficient replication or the inclusion of non-significant factors in the model. Therefore, further validation or refinement of the model may be necessary to improve its predictive reliability. However, the R^2 parameter meets the requirement with a value greater than 0.7, specifically 0.9535. The adequate precision value also satisfies the criteria, greater than 4 at 13.0951.



(a)



(b)



(c)

Figure 2. (a) Effect of Time and Solvent Volume on Yield, (b) Effect of Time and Power on Yield, (c) Effect of Solvent Volume and Power on Yield

Figure 2 illustrates the graphical effects of each extraction parameter (time, solvent volume, and microwave power) on lipid yield. The analysis was carried out using Design Expert software. The effect of extraction time is shown in Figures 2(a) and 2(b). These graphs indicate that yield increases with longer extraction time. This is because a longer duration of microwave exposure leads to greater energy absorption, converting electromagnetic energy into heat, which raises the temperature and enhances lipid yield [23].

The effect of solvent volume on yield is depicted in Figures 2(a) and 2(c). The graphs show that increasing the solvent volume leads to higher yield, as more solvent facilitates better diffusion of lipid components. A larger volume of solvent can penetrate deeper into the cell walls, increasing internal pressure. This pressure buildup may cause the cell walls to rupture, allowing more intracellular compounds to be extracted [24].

Figures 2(b) and 2(c) demonstrate that increasing microwave power also results in higher yield. Greater power intensifies microwave radiation, enhancing cell wall disruption and facilitating lipid release. However, power beyond the optimal level can negatively affect yield, as excessive energy may degrade the extracted lipids [23]. In this study, the highest yield was obtained at 300 W. At 450 W, the extracted lipids were likely damaged, leading to a decrease in lipid yield.

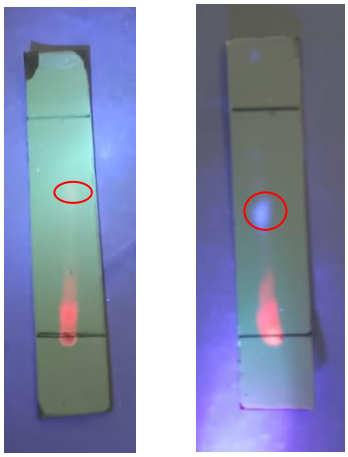
The lipid yields reported in previous studies were relatively low. In contrast, this study achieved a maximum yield of 15.8%. The higher yield obtained in this study can be attributed to the use of a different extraction method and the incorporation of DES during the pre-treatment process.

Lipid Characterization

Thin Layer Chromatography (TLC) is a method used to separate compounds based on differences in their distribution between a mobile phase and a stationary phase. The stationary phase is a polar silica gel plate, while the eluent consists of hexane-methyl acetate-acetic acid. TLC separates compounds based on polarity, where the polar stationary phase (silica gel) retains polar compounds more strongly, resulting in shorter migration distances compared to non-polar compounds [25].

TLC analysis aims to identify the chemical content of a sample based on its characteristic chromatogram [26]. A

study by Pesang et al. (2020) on *Ulva sp.* using maceration extraction produced 12 colored spots on the TLC plate, corresponding to pigments such as chlorophyll a, chlorophyll b, carotene, and pheophytin [2]. Another study by Lopez & Alexandra (2022), using hydrolysis-based extraction of *Ulva sp.*, applied TLC with succinate buffer and identified pigments such as galactomannan, cello-4, and laminarin-6 in the samples [27].



(a) (b)

Figure 3. (a) TLC Analysis Result of Sample 15, (b) TLC Analysis Result of Sample 5

TLC analysis in this study was conducted to qualitatively assess the presence of fatty acid compounds in the lipid extracts from *Ulva sp.* Based on Figure 3, it can be concluded that compound separation occurs over time depending on the solubility and retention of the compounds in the mobile and stationary phases. The solvent moves upward through the TLC plate via capillary action [28]. Figure 3(a) shows that the mobile phase produced an Rf value of 0.42, while the result for sample 5 in Figure 3(b) shows an Rf value of 0.38. The Rf value of fatty acid esters with higher polarity is typically around 0.82. Free fatty acids have various Rf values, such as stearic acid (Rf 0.59), palmitic acid (Rf 0.46), linoleic acid (Rf 0.25), linolenic acid (Rf 0.09), and oleic acid (Rf 0.04) [29]. The Rf values obtained in this study fall within the general range expected for fatty acids. However, specific identification of individual fatty acids was confirmed through GC-MS analysis.

Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

The lipid content extracted from *Ulva sp.* was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS). GC-MS is a quantitative analytical method to identify fatty acids or lipid derivatives in extracted samples. This technique determines the fragmentation patterns of saturated and unsaturated fatty acids, as well as the position of double bonds in the fatty acid structures [30]. GC-MS combines gas chromatography (GC), which separates compounds, and mass spectrometry (MS), which analyzes each detected peak.

The lipid extracts of *Ulva sp.* were dissolved in a chloroform-methanol solution (2:1) and analyzed for their lipid content. The samples selected for GC-MS analysis were those with the highest (sample 15) and lowest (sample 5) yields. GC-MS testing resulted in chromatograms with multiple peaks representing different detected compounds [31]. The analysis results include peak area and retention time for each component [32]. The height and intensity of each peak indicate the concentration of each compound detected in the sample [31].

The GC-MS chromatograms of *Ulva sp.* extracts are shown in Figure 4. The chromatogram for sample 15 revealed the presence of lipids, with the highest peak at a retention time of 41.893 min and a peak area of 5.07%. The chromatogram for sample 5 also showed lipid content, with the highest peak at a retention time of 41.033 min and a peak area of 16.23%. The differences in peak area percentages between the highest and lowest yield samples reflect the relative concentrations of compounds present in each sample [33].

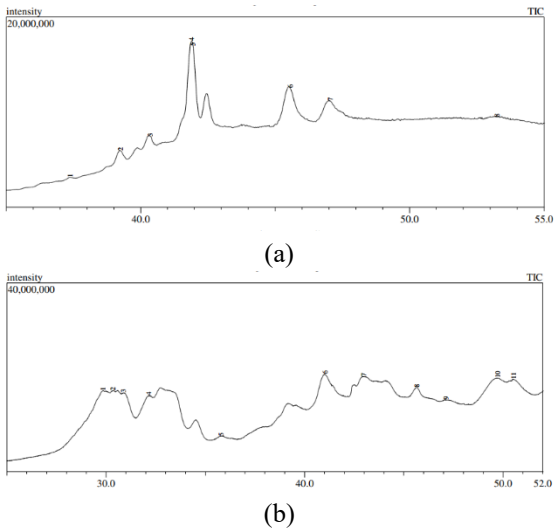


Figure 4. (a) GC-MS Chromatogram of *Ulva sp.* Extract Sample 15, (b) GC-MS Chromatogram of *Ulva sp.* Extract Sample 5

The results of lipid characterization using GC-MS are presented in Tables 5 and 6. The data show the various types and quantities of methyl esters produced from the transesterification reaction. Compounds with shorter hydrocarbon chains are detected earlier by the GC detector compared to those with longer chains. Short-chain esters are more polar than long-chain esters. This is because short carbon chains interact more weakly with the GC column, resulting in shorter retention times [30].

The GC-MS results for sample 15, presented in Table 5, show that the compound Pentadecanoic acid, 14-methyl-, methyl ester, a branched-chain saturated fatty acid, had the highest abundance based on peak area (33.86%). The identified compounds were not limited to fatty acids, as the chromatogram also detected other types of compounds. The fatty acids identified included palmitic acid, oleic acid, and palmitoleic acid. While palmitic acid (methyl palmitate) was also detected in significant quantities (26.33% total area), it was not the dominant component. Other fatty acids identified included oleic acid (20.72%) and palmitoleic acid (5.37%).

The GC-MS results for sample 5, shown in Table 6, identified several fatty acids, including palmitic acid, pentadecylic acid, margaric acid, stearic acid, linoleic acid, and enoic acid.

The TLC results for samples 15 and 5 indicated the presence of palmitic acid, as the Rf values of each sample (0.42 and 0.38, respectively) were close to the Rf value of

palmitic acid (0.46). Based on the GC-MS results, palmitic acid was also the dominant fatty acid in both samples, as indicated by the retention times and peak area percentages. The following section presents a comparison of the TLC and GC-MS results.

Table 5. Lipid Analysis Results of Sample 15

Peak#	R.Time	Area	Area%	Name
1	37.382	27428797	1.01	Tetradecanoic acid, methyl ester (CAS) Methyl myristate $$
2	39.252	142192574	5.21	NEOPHYTADIENE
3	40.362	204633047	7.50	NEOPHYTADIENE
4	41.893	138251887	5.07	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate
5	41.944	923528686	33.86	Pentadecanoic acid, 14-methyl-, methyl ester (CAS)
6	45.566	579926814	21.26	Hexadecanoic acid (CAS) Palmitic acid
7	47.057	565138749	20.72	9-Octadecenoic acid (Z)-, methyl ester (CAS) Methyl oleate
8	53.255	146530806	5.37	9-Hexadecenoic acid
		2727631360	100.00	

Table 6. Lipid Analysis Results of Sample 5

Peak#	R.Time	Area	Area%	Name
1	29.845	862719684	13.08	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate
2	30.344	202011761	3.06	Pentadecanoic acid, 14-methyl-. methyl ester (CAS)
3	30.887	417740289	6.33	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate
4	32.153	471201551	7.14	Heptacosanoic acid, methyl ester (CAS) Methyl Heptacosanoate
5	35.800	72530253	1.10	Pentadecanoic acid (CAS) Pentadecylic acid
6	41.033	1070640741	16.23	Heptadecanoic acid (CAS) Margaric acid
7	42.982	1076831911	16.33	11,14,17-Eicosatrienoic acid, methyl ester
8	45.668	663453034	10.06	Octadecanoic acid (CAS) Stearic acid
9	47.130	362548721	5.50	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS) Methyl linoleate
10	49.726	827380927	12.54	OCTADEC-9-ENOIC ACID
11	50.541	569034342	8.63	11,14,17-Eicosatrienoic acid, methyl ester
		6596093214	100.00	

Table 8 presents a comparison between sample 15 and sample 5. The yield of sample 15 was higher than that of sample 5 due to differences in operating conditions, particularly in solvent volume and microwave power. Sample 5 was extracted using 150 W of power and 60 mL of solvent. These conditions resulted in insufficient thermal energy to effectively extract lipids from the biomass. A lower solvent volume also limited the extent of component diffusion, reducing extraction efficiency.

Table 8. Comparison of TLC and GC-MS Test Results

		Sample 15	Sample 5
Yield (%)		15.8	2.11
TLC	Rf	0.42	0.38
GC-MS (highest peak)	Retention time	45.566	41.033
	Area	579926814	1070640741
	Area%	21.26	16.23
	Compound name	Palmitic acid	Margaric acid

The Rf value of sample 15 (0.42) was higher than that of sample 5 (0.38), as a higher yield in sample 15 led to a longer migration distance on the TLC plate. However, both Rf values are classified as palmitic acid, as they are close to the reference Rf value of 0.46. The GC-MS results also showed differences between the two samples, especially in palmitic acid content. The total palmitic acid content based on peak area in sample 15 was higher (26.33%) compared to sample 5 (19.41%).

Both samples were confirmed to contain palmitic acid. It is a saturated fatty acid with a 16-carbon long-chain structure [34]. Palmitic acid is one of the most important saturated fatty acids in biodiesel composition, while oleic acid, which contains one double bond, is a key unsaturated fatty acid. A high saturated fatty acid content in methyl esters is a good indicator of biodiesel's resistance to oxidation and contributes to a higher cetane number [35].

Fatty acids such as palmitic, oleic, palmitoleic, margaric, stearic, and linoleic acids meet this requirement [36]. A study by Kalavathy & Baskar (2019) on *Ulva lactuca* using autoclave followed by ultrasonication and analyzed via GC-MS identified long-chain fatty acids such as pentadecanoic acid, palmitic acid, octadecanoic acid, decanoic acid, elaidic acid, cholesterol, and other saturated

and unsaturated fatty acids [48]. This study demonstrates that *Ulva sp.* contains fatty acids such as palmitic, oleic, stearic, and linoleic acids, indicating that *Ulva sp.* lipids contain a diverse composition of free fatty acids (FFAs), including both saturated and unsaturated types [37].

In addition to enhancing lipid yield, the combination of MAE and DES pretreatment offers scalability and energy efficiency benefits. MAE provides rapid heating and reduced solvent usage compared to conventional methods, while DES, particularly those based on urea and $ZnCl_2$, can be synthesized from inexpensive and recyclable components. These features make the process promising for industrial-scale applications, although further techno-economic assessments are needed.

The results of the GC-MS analysis confirmed the presence of several major fatty acids in Ulva sp., which are dominated by palmitic, oleic, and palmitoleic acids. These findings demonstrate that combining aDES pretreatment and MAE can effectively release FFAs from macroalgal biomass. The detection of FFAs in transesterified samples also indicates that some fatty acids may have existed in their free form before derivatization. Palmitic acid and oleic acid align with the fatty acid profile that is desirable in biodiesel production. Saturated fatty acids such as palmitic acid enhance oxidative stability and cetane number, while monounsaturated fatty acids like oleic acid improve cold flow properties [38].

These lipids extracted from *Ulva sp.* may serve as a viable feedstock for renewable biofuel. Future research should consider extending this extraction method to other macroalgae species and evaluating its techno-economic feasibility for large-scale production.

Conclusion

The results of lipid extraction from *Ulva sp.* showed that the highest yield obtained was 15.8% under the operating conditions of 20 min extraction time, 75 mL solvent volume, and 300 W of microwave power. The lowest yield, 2.11%, was achieved under 20 min, 60 mL of solvent, and 150 W of power. The yields obtained in this study were higher than those reported in previous literature, likely due to the use of DES, which enhances lipid extraction efficiency. GC-MS analysis of the sample with the highest yield identified fatty acids such as palmitic acid, oleic acid, and palmitoleic acid. These findings suggest that lipids extracted from *Ulva sp.* using DES-MAE can be further explored for applications in biodiesel formulation or as a source of pharmaceutical-grade fatty acids.

Author's Contribution

Meta Fitri Rizkiana: Designed the research concept, developed the methodology, and supervised the overall research process. Afrila Tutut Dwijati Lestari: Conducted the extraction experiments and collected the data. Irdatus Sholeha: Wrote and edited the results and discussion sections, and prepared the manuscript for submission. Bekti Palupi: Conducted GC-MS and TLC characterization of lipid samples. Ditta Kharisma Putri: Performed statistical analysis and interpreted the optimization results. Istiqomah: Assisted in data tabulation and preparation of research tables and figures. Helda Wika Amini: Supported sample preparation and TLC testing procedures. Boy Arief Fachri: Helped in literature review and formatting of references and citations.

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