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The Physicochemical Characteristics of Crude Oil Extract *Spirulina* sp. by Osmotic-Shock Extraction Method

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Abstract: *Spirulina* sp. is a potential microalgae. One of the potentials that continue to be developed, such as edible oil. Various extraction methods can obtain Spirulina oil content, i.e., the osmotic shock method as a green extraction method. Each method used can affect the fatty acid components. Not many studies have reported the physicochemical characteristics of oil extract *Spirulina* using the osmotic-shock method. This study analyzes the physicochemical characteristics of crude oil extract *Spirulina* sp. extracted using the osmotic-shock method. The study used a simple, completely randomized design with treatment extraction times of 60, 90, and 120 minutes. The data were analyzed using analysis of variance and Duncan's test (α5%). The results showed that the treatment level of 60 minutes produced the lowest physicochemical characteristics and met the International Fish Oil Standards, where peroxide value 1.49±0.0089 mEq/Kg, iodine value 71.37±0.0035 g I_2/g, saponification value 502.69±0.0069 mg KOH/g, acid value 0.049±0.0011 mg KOH/g, and Free Fatty Acid 0.0102±0.0003%. The total concentration of Saturated Fatty Acids is 36.18%, monounsaturated fatty Acids 7.72%, and polyunsaturated fatty Acids 49.41%. The fatty acid components are oleic acid/ω-9, linoleic acid/ω-6, linolenic acid/ω-3, eicosapentaenoic acid, and docosahexaenoic acid. The presence of EPA and DHA in the crude oil extract of *Spirulina* sp. showed potential for development as edible oil. The physical profile of *Spirulina* sp. oil extract obtained by the osmotic-shock method meets International Fish Oil Standards.

Keywords: Docosahexaenoic Acid; Edible Oil; Eicosapentaenoic Acid; Green Extraction; Microalgae.

Introduction

Spirulina sp. is one of the potential microalgae that continues to be developed. Since *Spirulina* sp. contains high amounts of primary metabolites, such as carbohydrates, lipids, and proteins. *Spirulina* flour contains 13.63% carbohydrates, 1.27% lipids, and 71.90% protein [1]. In addition to primary metabolites, *Spirulina* also contains secondary metabolites, such as flavonoids and phycocyanin [2], which have various biological activities, such as antimicrobial, antioxidant, anticancer, and cholesterol-lowering effects [3]. In addition to having biological activity, the compounds contained in *Spirulina* have extensive use in the industrial sector, such as developing lipids into edible oil. An extraction process using various types of solvents, either single solvents or combinations of solvents, is performed to obtain the metabolite compounds in Spirulina.

Extraction is the first step to separating the targeted natural product from the raw material. The conventional extraction method has been widely used in the maceration method. However, the maceration method has weaknesses, requiring a long extraction time and large amounts of solvent [4]. The advantages of the extraction method with the principle of cell disruption are that it is pretty effective against cell walls with considerable stiffness, can overcome high cell wall stiffness, dispersion of intracellular compounds directly into the medium, active cooling can minimize degradation of thermolabile compounds, high reproducibility, and optimization of processes that are efficient and also relatively easy [5]. So, developing a non-conventional extraction method based on the cell disruption method is necessary. There are several types of extraction using the cell-disruption principle method, such as ultrasound, chemical or enzymatic treatment, thermal or osmotic shock, and mechanical action such as high pressure and bead mills [6].

One of the extraction methods based on mechanical extraction of disrupted cells that is still not widely known to extract metabolites such as lipids from *Spirulina* is an osmotic shock. The osmotic-shock method is a novelty extraction method induced when there is a change in concentration around the cell. The cell wall breaks due to permeation to reach equilibrium with the medium. This allows the extraction of lipids because they are no longer contained in the cells [7].

The method of osmotic shock extraction has not been studied in the extraction of crude oil from *Spirulina*. This study analyzes the physicochemical characteristics of crude oil extract *Spirulina* sp. extracted using the osmotic-shock method.

Research Methods

Materials

The materials for this research were *Spirulina* powder, HCl (Sigma-Aldrich), n-Hexane (Sigma-Aldrich),

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ddH_2O (DSBio), filter paper Whatman No. 1 (Sigma-Aldrich), KOH (Sigma-Aldrich), NaOH (Sigma-Aldrich), chloroform (Sigma-Aldrich), glacial acetic acid (Sigma-Aldrich), potassium iodide (Sigma-Aldrich), starch solution (Sigma-Aldrich), sodium thiosulfate (Sigma-Aldrich), Wijs reagent (Merck), and ethanol (Sigma-Aldrich).

Extraction of Oil from Microalgae *Spirulina* sp.

Prepared *Spirulina* sp. powder, 100 g in Erlenmeyer, then added 100 mL of 5 M HCl. They were incubated in a water bath at 50°C with a time level of 60, 90, and 120 min. Separate the filtrate and residue with filter paper. Whatman No. 1. Purified the filtrate with 32 mL of n-Hexane & 60 mL of ddH_2O until two liquid-liquid layers are formed. Evaporation was carried out on the n-Hexane layer to evaporate the solvent and obtain a crude extract of *Spirulina* oil. Neutralized with 10 mL of 5 M NaOH, then distilled crude oil extract *Spirulina* sp—analysis of %yield of crude oil extract [8].

Analysis Quality of Crude Oil Extract of *Spirulina* sp.

A titrimetric method was used to analyze the quality of crude oil extract of Spirulina sp. [8]. The parameters for determining the quality of *Spirulina* sp. crude oil extract, namely, peroxide value, iodine number, saponification value, acid value, and %free fatty acid (FFA) from acid value.

Analysis Fatty Acid Components of Crude Oil Extract *Spirulina* sp.

GC-MS was used to identify the fatty acid components of the crude oil extract of Spirulina sp. The specifications of the GC-MS are Agilent Technologies 5973 N, Capillary Column DB 5 with length 60 mm 0.25 µm, volume of injection 2µL, inlet temperature 290°C, aux temperature 290°C, program temperature 70°C (15°/min) to 290°C (20 min), mode of gas flow constant, Helium as carrier gas; flow rate of 1 mL/min. A chromatogram was obtained, and the mass spectrum of the unknown component was compared with the spectrum of the known components stored in the WILEY 10 library [9].

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) and Duncan's post hoc test (α 5%) using Minitab version 26.

Results and Discussion

The %Yields of Crude Oil Extract *Spirulina* sp.

In this study, we extracted oil from the dried biomass of Spirulina sp. using the cell disruption method at different levels. The %yields of crude oil extract *Spirulina* sp. at different level times of extraction are provided in Table 1, which showed that the use of HCl as an osmotic agent in this study successfully improved the %yields of crude oil extract *Spirulina* sp. compared to other cell disruption methods such as ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). Djamaludin and Chamidah [10] reported %yields of UAE 2.15% and MAE 1.11% for the crude oil extract *Spirulina* sp. The disrupted cell wall of *Spirulina* sp. readily released its intracellular components. The total amount of oil extracted from *Spirulina* sp. has been adopted to indicate the efficiency of osmotic shock as a cell disruption method. Cell disruption can affect the amount of oil extracted [11].

In addition to the extraction method, the concentration of solvent used can also affect the %yields of crude oil extract *Spirulina* sp. Rachmaniah *et al*. [12] reported that using hydrochloric acid (HCl) as an osmotic agent with different concentrations gave different %yields. *Spirulina* sp. oil extraction result using different concentrations gave different %yields of oil: 3 M HCl was 25.706%, and 5 M HCl was 71.236%. The cell walls of *Spirulina* sp. contained complex polysaccharides. The acidic conditions can completely hydrolyze constituent polysaccharides.

The cell wall of microalgae is composed of sulfated polysaccharides-gel with acidic characteristics and lacks cellulose microfibril components [13]. This sulfated polysaccharide can be wholly hydrolyzed in strongly acidic solvents. This separates the non-polar phase from the polar phase. Laurens et al. [14] reported that up to 97% w/w of lipids can be extracted using dilute acid. This completely hydrolyzes the cell walls of the microalgae. Oil extraction was performed using the osmotic-shock method of cell disruption using dried biomass of *Spirulina* sp. HCl solution as a penetrant is more effective for oil extraction. Also, the HCl solution has a higher osmotic pressure than the CH_3COOH solution because the HCl solution contains more particles [5].

Table 1. The %yields of crude oil extract of *Spirulina* sp. at different level times of extraction

No.	Time (min.)	%Yields of crude oil extract of *Spirulina* sp.
1	60	5.20 ± 0.03^a
2	90	5.39 ± 0.05^a
3	120	5.57 ± 0.09^a

The average value with equal letters in each column shows that it is not significantly different based on Duncan's Test.

The Quality of Crude Oil Extract *Spirulina* sp.

Oil oxidation is an essential parameter that significantly affects the quality and value of all oil grades. Fatty acyl hydroperoxides are formed during oil oxidation because unsaturated fatty acids react with molecular oxygen [15]. Oil oxidation also alters organoleptic parameters, increases toxic compounds, and reduces food quality [16]. In this study, we determined the physicochemical properties of crude oil extract *Spirulina* sp. by analyzing the peroxide, iodine, saponification, acid, and percentage of free fatty acid components.

Peroxide Value

Peroxide Value (PV) is one of the physicochemical characteristics used to determine the quality of edible oils. The PV indicator shows the amount of peroxide in mg of active oxygen per 1000 g of oil. [10]. PV indicates the level

of damage in terms of hydroperoxide levels in the oil, which are the main products of the oxidation process. The higher the peroxide value, the lower the quality of the oil. PV can be considered an index during the early oxidation of oil. Oxidation occurs when unsaturated fatty acids react with oxygen to form hydroperoxide components. This system can continue unexpectedly while there are assisting elements, including temperature and light (17). Many studies have shown that peroxides oil may affect many degenerative diseases [18] [19] [20]. The results of statistical analysis using one-way ANOVA showed that the PV of crude oil extract Spirulina sp. was significantly different ($p<0.05$) for each extraction time (Table 2). This research found that the PV of crude oil extract *Spirulina* sp. is 1.49±0.0089 up to 3.67±0.0082 mEq/Kg and still met the IFOS if the PV is higher than 5 meq/Kg, which indicates the oxidizing of the oil.

Iodine Value

Iodine Value (IV) is also a crucial parameter of edible oil, indicating the proportion of unsaturated fatty acids. The iodine value of crude oil extract *Spirulina* sp. is 71.37±0.0039 up to 108.89±0.0095 g I_2/g and still met the IFOS. The results of statistical analysis using one-way ANOVA showed that IV of crude oil extract Spirulina sp. were significantly different ($p<0.05$) for each extraction time (Table 2). This finding is lower than that of Djamaludin and Chamidah (10) that IV of crude oil extract Spirulina sp. was 116.19 g I2/g using UAE methods. The higher the iodine number, the more double bonds can be added and the higher the degree of unsaturation. Unsaturated fatty acids in edible oils take up iodine and form saturated compounds. The amount of iodine ingested indicates the number of double or unsaturated bonds. A higher iodine value means lower oxidation stability, affecting the quality of edible oils [21].

Saponification Value

The Saponification Value (SV) rate is the number of mg of KOH required to saponify 1 g of oil. SV determines the average size of fatty acids present in the oil. This depends on the molecular weight and the percentage of fatty acid components. SV increases as the oil contains more saturated fatty acids such as C14:0, C16:0, and C18:0, determining the length of carbon chains in the oil. Low molecular weight oils have a higher SV than high molecular weight oils. The presence of unsaponifiable compounds in the oil can affect SV in oil, reducing the oxidative power of unsaturated fatty acid bonds [22]. The SV of crude oil extract *Spirulina* sp. was 502.69±0.0069 up to 523.51±0.0098 mg KOH/g. The results of statistical analysis using one-way ANOVA showed that the SV of crude oil extract Spirulina sp. were significantly different ($p<0.05$) for each extraction time (Table 2).

Acid Value

Acid Value (AV) is the number of mg of KOH required to neutralize the free fatty acids in 1 g of oil. A low acidity indicates that the oil is less sour. AV is an essential parameter of the physicochemical characteristics of oils. Free fatty acids in oil indicate past lipase activity, hydrolytic effects, or oxidation. AV is used to determine the extent to which glycerides in oil have been broken down by lipase or light and heat. High AV values indicate poor-quality fish oil [23]. Fats are generally in the form of triglycerides but undergo hydrolysis to free fatty acids. AVs are closely related to free fatty acids. The higher the acid number, the higher the free fatty acid value, which reduces the oil quality [24]. The AV of Spirulina sp. crude oil extract was determined to be 0.049±0.0011 up to 0.081±0.0021 mg KOH/g. The results of statistical analysis using one-way ANOVA showed that the AV of crude oil extract *Spirulina* sp. were significantly different ($p<0.05$) for each extraction time (Table 2).

Table 2. Physicochemical Characteristics of Crude Oil Extract *Spirulina* sp.

No	Parameters	Time (minutes)			Max. Standard
		60	90	120	
1.	PV	1.49±0.0089[a]	2.16±0.0089[b]	3.67±0.0082[c]	5 mEq / Kg
2.	IV	71.37±0.0035[a]	83.06±0.0069[b]	108.89±0.0095[c]	155 g I_2 / g
3.	SV	502.69±0.0069[a]	513.43±0.0082[b]	523.51±0.0098[c]	565 mg KOH / g
4.	AV	0.049±0.0011[a]	0.062±0.0017[b]	0.081±0.0021[c]	3 mg KOH / g
5.	FFA	0.0102±0.0003[a]	0.0257±0.0020[b]	0.0455±0.0006[c]	1.50 %

Based on Duncan's Test, the average value with unequal letters in each row is significantly different.

Fatty Acid Components of Crude Oil Extract *Spirulina* sp.

Analysis by GC-MS has high specificity when analyzing the fatty acid components contained in fats and oils and the molecular weight of each fatty acid [9]. The details of the fatty acid content analysis of crude oil extract Spirulina sp. are shown in Table 3.

The total concentration of Saturated Fatty Acid (SFA) is 36.18%, Monounsaturated Fatty Acid (MUFA) is 7.72%, and Polyunsaturated Fatty Acid (PUFA) is 49.41%. The fatty acid components contained in the crude oil extract *Spirulina* sp. extracted by osmotic shock method such as butyric acid, lauric acid, tridecanoic acid, myristic acid, palmitic acid, heptadecanoic acid, stearic acid, palmitoleic acid, heptadecanoic acid, oleic acid/ω-9, linoleic acid/ω-6, linolenic acid/ω-3, eicosapentaenoic acid (EPA)/ω-3, and docosahexaenoic acid (DHA)/ω-3. The fatty acid components obtained in this study are not much different from those reported by Djamaludin and Chamidah [9], which extracted *Spirulina* sp. using the UAE and MAE methods. Where the results of the extraction with both methods obtained types of fatty acids such as butyric acid, lauric acid, tridecanoic acid, myristic acid, palmitic acid, heptadecanoic acid, stearic acid, palmitoleic acid, heptadecanoic acid, oleic acid/ω-9, and linoleic acid/ω-6.

Table 3. The fatty acid components of crude oil extract *Spirulina* sp.

Saturated Fatty Acid (SFA)	Result
Butyric acid (C4:0)	0.15%
Lauric Acid (C12:0)	0.16%
Tridecanoic Acid (C13:0)	0.29%
Myristic Acid (C14:0)	0.39%
Palmitic Acid (C16:0)	33.35%
Heptadecanoic Acid (C17:0)	0.54%
Stearic Acid (C18:0)	1.30%
Total of SFA	36.18%
Mono-Unsaturated Fatty Acid (MUFA)	**Result**
Palmitoleic Acid (C16:1)	3.36%
Heptadecanoic Acid (C17:1)	0.19%
Oleic Acid/ω-9 (C18:1)	4.17%
Total of MUFA	7.72%
Polyunsaturated Fatty Acid (PUFA)	**Result**
Linoleic Acid/ω-6 (C18:2)	18.83%
Linolenic Acid/ω-3 (C18:3)	17.00%
Eicosapentaenoic Acid (EPA)/ω-3 (20:5)	7.70%
Docosahexaenoic Acid (DHA)/ω-3 (22:6)	5.88%
Total of PUFA	49.41%

The highest fatty acid for SFA was palmitic acid (C16:0) in 33.35%, the highest fatty acid for MUFA was oleic acid/ω-9 (C18:1) in 4.17%, and the highest fatty acid for PUFA was linoleic acid/ω-6 (C18:2) in 18.83%. Gorjzdadeh *et al*. [25] also reported that the maximum amount of total fatty acids of *Spirulina* sp. belonged to palmitic acids (C16:0) with 30.1% of total fatty acids respectively. The MUFA showed that the oleic acid was the higher amount in *Spirulina* sp., and the PUFA was linoleic acid (C18:2) (Omega 6), revealing the maximum percentage in Spirulina sp. with 18.8%.

In addition, this study's results also showed omega-3 fatty acids such as EPA and DHA with levels of 7.70% and 5.88%, respectively. Widianingsih *et al.* [26] reported that the fatty acid component of oil extract *Spirulina platensis* contains omega-3 EPA 0.22±0.04. Raya *et al*. [27] also noted the presence of omega-3 such as DHA and EPA in *Spirulina platensis* 72,345 mg/g and 331.07 mg/g dry weight. The presence of EPA and DHA in crude oil extract *Spirulina* sp. extracted by osmotic shock method indicates that *Spirulina* sp. may be developed as an edible oil with many benefits and various biological activities, such as anti-inflammatory [28], increase immune function, [29] and EPA and DHA also help prevent degenerative diseases [30].

Conclusion

Based on the research showed that the treatment level of 60 minutes produced the lowest physicochemical characteristics and met the International Fish Oil Standards, where peroxide value 1.49±0.0089 mEq/Kg, iodine value 71.37±0.0035 g I_2/g, saponification value 502.69±0.0069 mg KOH/g, acid value 0.049±0.0011 mg KOH/g, and Free Fatty Acid 0.0102±0.0003%. Saturated Fatty Acids are 36.18%, Monounsaturated Fatty Acids 7.72%, and Polyunsaturated Fatty Acids 49.41%. The fatty acid components are oleic acid/ω-9, linoleic acid/ω-6, linolenic acid/ω-3, eicosapentaenoic acid, and docosahexaenoic acid. The fatty acid components are butyric acid, lauric acid, tridecanoic acid, myristic acid, palmitic acid, heptadecanoic acid, stearic acid, palmitoleic acid, heptadecanoic acid, oleic acid/ω-9, linoleic acid/ω-6, linolenic acid/ω-3, EPA/ω-3, and DHA/ω-3.

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