Improvement of lipid yield from microalgae *Spirulina platensis* using ultrasound assisted osmotic shock extraction method

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**Abstract.** Microalgae *Spirulina sp.* has been identified as potential source of natural food supplement and food colorant. The high water content of microalgae (70-90%) causes an obstacle in biomass dehydration which requires large amounts of energy, eventually damaging the lipid in the microalgae. Therefore, the lipid must be extracted by using a suitable method which complies to wet biomass conditions. One of the methods is applying osmotic shock. This study was aimed to investigate the influence of osmotic agent (NaCl) concentration (10-30%) and extraction time (20-50 min) on yield of lipid and also to determine the optimal conditions in the extraction process through response surface methodology. The extraction was conducted at a temperature of 40°C under ultrasound frequency of 40 kHz. The result showed that the optimum yield lipid obtained was 6.39% in 16.98% NaCl concentration for 36 minutes 10 seconds.

**Keywords:** extraction; lipid; microalga; osmotic shock; ultrasound.

1. **Introduction**

Food is not only used as energy and nutrient source, but also can improve the immune system for our body. Functional food can be produced by adding ingredients which have special function for our health into the food product. Most people living in developing countries are in the state of starvation due to limited source and access to minerals, proteins, vitamins, and nutritious food [1]. The lack of functional compound can be overcome by finding alternative compounds from natural sources, one of which is microalgae. Microalgae is the most efficient plant in capturing and harnessing the solar energy for photosynthesis, fast growth and high productivity [2].

*Spirulina sp.* is becoming one of the most promising microalgae to be cultivated in Indonesia due to considerable potential as food source and natural coloring agent. It is because *Spirulina sp.* is the highest protein source from microbe which is 46-63% of its dry weight and also high potential compared to soy (40% dry weight) [3,4]. This microalgae has high nutritional values which are: 63% protein content, 8-14% carbohydrate and the rest are, vitamin, mineral also pigments that consists of: chlorophyll, carotene, xanthophyll and phycocyanin [4].

Components of microalgae consist of three main parts which are lipid, protein, and carbohydrate. The important stage in producing lipid from microalgae is extraction [4]. The high water content of
microalgae (70-90%) requires process of biomass dehydration so that the lipid contained in the microalgae can be extracted optimally. The process of dehydration needs a large amount of energy and potentially damages the lipid contained in the microalgae. This is the reason why the use of direct extraction methods by using wet biomass microalgae as the most interesting to develop [5].

Lipid accumulated in the microalgae has tendency to increase and improve if the organism was faced with stress [6]. Lipid extraction from wet biomass can be done efficiently by doing cell disruption, for example osmotic shock. The osmotic method is chosen considering Spirulina sp. is a microscopic multicellular organism which has a semipermeable membrane that is vulnerable to the changes of osmotic pressure [7]. Osmotic shock is the sudden change of soluble substance concentration around the cell by adding osmotic agent solution which causes a rapid change of water flow through the cell’s membrane. Osmotic agent is a substance which can produce osmotic pressure that passes through the semipermeable membrane of the cell [5]. The amount of osmotic pressure is inversely proportional to the weight of osmotic agent’s molecule. The amount of soluble substance in the solution will affect the osmotic pressure on microalgae’s cell, as a result, water from inside the cell move to the solution and finally shrinking lysis happen. The cell’s lysis will extract more lipid from inside the cell [6].

The osmotic shock method has a weakness which is the requirement of relatively long soaking time [8]. To deal with this problem, the ultrasound assisted extraction method can be used to speed up the cell disruption process [9]. The process of extraction is influenced by many factors such as particle size, type of soluble and solvent substance, temperature, stirring and extraction time [5]. Optimizations are needed in order to obtain the optimum variables during the extraction process one of them is by using Response Surface Methodology (RSM) and Central Composite Design (CCD) optimization method. RSM is a technique used to find out the value of the best respond while CCD is the research design used in Respond Surface Methodology [10]. This study was aimed to investigate the effect of NaCl concentration as osmotic agent and extraction time to lipid yield. The aim was also to determine the optimal condition for lipid extraction by using ultrasound assisted osmotic shock method.

2. Materials and Methods

2.1. Materials

Spirulina platensis wet biomass was provided by CV. Neoalgae (Sukoharjo, Central Java, Indonesia), n-hexane (Merck), NaCl (Merck) and aquades from Membrane Research Centre (MerC) Laboratory Diponegoro University.

2.2. Osmotic shock

Lipid from wet biomass extracted by using osmotic shock method [5, 11]. NaCl solution was used as an osmotic agent with concentration variations 10, 20 and 30%. Twenty grams of Spirulina sp. wet biomass were added to 50 ml of NaCl solution. The sample was left for 1 hour and then filtered to get the extract.

2.3. Ultrasound assisted lipid extraction

Lipid were extracted by using n-hexane solvent method with ultrasound assisted extraction (UAE) [11, 12]. n-hexane solvent was added to the sample with variation of solvent to biomass ratio 10:1. Then the sample was agitated for an hour to make it well mixed. The mixture was then sonicated with of 20, 35 and 50 minutes time variation. The mixture between residue and supernatant was separated by using filter paper and vacuum pump. The supernatant was evaporated with rotary vacuum evaporator in 60°C temperature to separate lipid from n-hexane solvent. Lipid yield was determined by using the following formula (Eq.1).

\[
\text{Total Lipid} = \frac{\text{weight lipid produced}}{\text{total weight of biomass}} \times 100\%
\]
2.4. Optimization with RSM-CCD
Response surface method was used to observe the effect of NaCl concentration treatment and extraction time. Two independent variables of the experiment, named NaCl solution concentration \( (X_1) \) and extraction time \( (X_2) \) were used as controlled factors. Lower, upper, and center point from the design where +1 as high level, -1 low level, \( \alpha = 2^n/4 \) \( (n=\) number of variables or factors) as star point, and 0 is the center point. Star point was added into the design to get estimation curve in the model [10]. Based on the type of experimental design used, 12 experiments were required. In this study Design Expert 7.0.0 (Trial Version State-Ease., MN) program was used for statistical data processing. Design of the experiment shown in Table 1 and Table 2.

Table 1. Factor of the experimental formulation

| Faktor                        | Star Point (Low) | Low Level (-1) | High Level (+1) | Star Point (High) |
|-------------------------------|------------------|----------------|-----------------|------------------|
| NaCl concentration (%)        | 5.86             | 10             | 30              | 34.14            |
| Extraction time (min)         | 13.79            | 20             | 50              | 56.21            |

Table 2. Central Composite Design trial table

| Run | NaCl (%) | Time (min) | Lipid Yield (Y) |
|-----|----------|------------|-----------------|
|     | X1       | X2         | Experiment     | Prediction     |
| 1   | 10.00    | 50.00      | 3.92           | 3.63           |
| 2   | 5.86     | 35.00      | 4.51           | 4.53           |
| 3   | 34.14    | 35.00      | 1.53           | 1.80           |
| 4   | 20.00    | 35.00      | 5.91           | 6.24           |
| 5   | 20.00    | 13.79      | 2.63           | 2.46           |
| 6   | 30.00    | 20.00      | 0.92           | 0.90           |
| 7   | 10.00    | 20.00      | 4.34           | 4.49           |
| 8   | 20.00    | 56.21      | 3.12           | 3.58           |
| 9   | 20.00    | 35.00      | 6.83           | 6.24           |
| 10  | 30.00    | 50.00      | 3.82           | 3.36           |
| 11  | 20.00    | 35.00      | 6.41           | 6.24           |
| 12  | 20.00    | 35.00      | 5.82           | 6.24           |

3. Result and Discussion

3.1. The effect of NaCl concentration to lipid yield
The extraction of lipid was conducted through osmotic shock method using wet biomass (water content ≥80%). Osmotic shock is the sudden change of soluble substance concentration around the cell by adding osmotic agent solution which cause rapid change of water that flow through the cell’s membrane. Osmotic agent can be either electrolyte or non-electrolyte such as NaCl, CH₃COONa, Na₂SO₄, glucose, sucrose and lactose [8].

Osmotic pressure is inversely proportional to weight of molecule. NaCl is an electrolyte solution with a small molecule weight (58.44 g/mol), Ionic osmotic agent is more effective for lipid extraction compared to non-ionic osmotic agent [5]. This is because electrolyte solution has more particles than...
non-electrolyte in the same concentration which cause the osmotic pressure of electrolyte solutions is higher than non-electrolyte [13]. The use of NaCl as osmotic agent successfully improved the amount of yield lipid from *Spirulina sp.* wet biomass to almost twice compared to conventional method (3.5%) [5].

![Figure 1](image)

**Figure 1.** Effect of NaCl concentration to lipid yield.

The effect of NaCl as osmotic agent in the extraction is displayed in Figure 1. The results of the study is consistent with the initial hypotheses that osmotic shock can improve the extraction of lipid yield. In this study three different concentrations of NaCl were tested and the result was the optimum amount of lipid obtained through osmotic shock method was 6.83% in 20% concentration of NaCl solution. Yield lipid decreases as the use NaCl concentration more than 20%, this happened because not all NaCl dissociates. Even some of the Na\(^+\) and Cl\(^-\) ions quickly convert back to NaCl and this is more likely to occur at larger concentrations [14]. This NaCl settling will reduce the dissolved ions and the Van't Hoff NaCl factor which further affects the osmotic pressure. In addition, the higher concentration of salt will also increase the consumption of salt and may cause environmental effect when released [5].

### 3.2. The effect of extraction time to lipid yield

The amount of extraction time shows exponential increase of yield lipid, as the time goes. At 20 minutes of extraction there was gradual increase of yield lipid until 35 minutes later it became relatively constant (Figure 2). This increase of yield lipid shows that ultrasound irradiation affect the numbers of cell disruption during extraction process. The forming of micro-bubble due to the effect cavitation that gave off large amount of energy to the surrounding biomass that cause disruption of cell wall [15]. The disruption of cell wall will release lipid from microalgae’s cell. The amount of time for extraction, can increase the transfer rate of the mass but on the other side, continuous ultrasound radiation may cause the solvent become saturated and thus will decrease the lipid yield [16]. The condition occurs because the solvent is not able to dissolve the solute, or the amount of solute has exceeded the capacity of the solvent. In addition, a decrease in extraction yield after 35 minutes occurred due to lipid damage after a balanced condition was achieved with longer extraction time.
Ultrasound assisted extraction method is very positive for intensifying the extraction process. Conventional extraction with soxhletation method using n-hexane solvent for 2 hours was done as a comparison. Yield lipid obtained from sonication (6%) for 30 minutes was almost twice more than yield lipid of conventional extraction (3.5%). Ultrasonic wave can facilitate expansion cell and the dissolving of the components which were caused by the cell wall pores expansion. Bigger pores expansion will increase the transfer rate of mass so it will increase efficiency and reduce extraction time [17].

3.3. Optimization with RSM-CCD

The suitable model for respond is quadratic model with 0.0002 of p value which shows that the margin of error is less than 5% so the quadratic model has significant influence on yield lipid respond. Table 3 showed the quadratic selection analysis of the lipid yield.

| Source       | Sum of Squares | df | Mean Square | F Value | p-value | Prob > F |
|--------------|----------------|----|-------------|---------|---------|----------|
| Model        | 37.87          | 5  | 7.57        | 34.83   | 0.0002  | (Significant) |
| A- Concentration | 7.48          | 1  | 7.48        | 34.39   | 0.0011  |
| B- Time      | 1.26           | 1  | 1.26        | 5.79    | 0.0529  |
| AB           | 2.76           | 1  | 2.76        | 12.67   | 0.0119  |
| A²           | 15.09          | 1  | 15.09       | 69.40   | 0.0002  |
| B²           | 16.55          | 1  | 16.55       | 76.11   | 0.0001  |
| Residual     | 1.30           | 6  | 0.22        |         |         |          |
| Lack of fit  | 0.64           | 3  | 0.21        | 0.97    | 0.5097  |
| Pure error   | 0.66           | 3  | 0.22        |         |         |          |

Deviation Standard = 0.47
R² = 0.9667
R² Adjusted = 0.9389
R² Predicted = 0.8533

Quadratic model has 0.47 standard deviation with R² = 0.9667 and 0.8533 of predicted R² almost close to 1. The value of coefficient X₁² and X₂² are negative, which indicate the existence of maximum
stationery point from the surface respond or open bottomed graphic [18]. The polynomial equation is as follows.

\[ Y = (-3.77387) + 0.32390X_1 + 0.41608X_2 - 0.015356X_1^2 - 0.00714X_2^2 + 0.00553X_1X_2 \]  (2)

where:
- \( Y \) = yield lipid respond (%)
- \( X_1 \) = NaCl concentration (%)
- \( X_2 \) = extraction time (minutes)

![Figure 3](image.png)

**Figure 3.** Interaction of NaCl concentration and time to lipid yield.

The Equation 2 is the actual equation needed to find out yield lipid respond which will be obtained if the value of needed variables are different. Interaction graphic between variables over yield lipid is shown in Figure 3. \( P_{\text{value}} \) regression of 0.000 is smaller than the degree of significance \( \alpha = 5\% \). This means that all the variables (\( X_1 \) and \( X_2 \)) have a significant effect in the model, both for linear and quadratic models [18]. The quadratic factor of NaCl concentration (\( X_1^2 \)) and quadratic time of extraction (\( X_2^2 \)) turned out to have a negative effect on the lipid yield response. Overall yield of lipid yields ranged from 0.92-6.83\%, which varied according to process variables. Increased extraction time gave the most significant effect on the yield of lipid yield. Figure 3 shows that an increase in NaCl concentration from 20-35 min tends to increase lipid yield. However, a longer time leads to a decrease in lipid yield. The longer the contact time, the more Na\(^+\) ions will move to the plasma membrane, thus reducing the osmotic pressure present [14].

From optimization procedure the optimum value for NaCl concentration (\( X_1 \)) and extraction time (\( X_2 \)) were obtained. By using this optimal parameters, yield lipid was recalculated with the equation 2. Additional experiment in optimal condition was conducted to confirm the result of prediction. Optimization shows that the optimal condition for extraction is \( X_1 = 16.98\% \) and \( X_2 = 36 \) minutes 10 seconds and produce 6.39\% yield lipid. The result show that extraction can be conducted with less amount of time compared to conventional method.
4. Conclusion

Extraction of lipid from microalgae by using ultrasound assisted osmotic shock method was studied for *Spirulina sp.* The result of extraction was then optimized using respond surface methodology. The result of the study shows that NaCl concentration as osmotic agent and extraction time has significant influence on yield lipid. Optimal condition was achieved by using 16.89% concentration NaCl and conducted for 36 minutes 10 seconds. The amount of yield lipid obtained in optimal condition was 6.39%.

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