

EXTRACTION OF EICOSAPENTAENOIC ACID FROM *Nannochloropsis gaditana* USING SUB-CRITICAL WATER EXTRACTION

(Pengekstrakan Asid Eikosapentaenoik daripada *Nannochloropsis gaditana* dengan Menggunakan Kaedah Air Sub-kritikal)

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Abstract

Microalgae had been utilized for biofuel production due to the presence of high lipid concentrations in the past year. However, the current interest is to convert their lipids to produce value added products such as omega-3. Various types of microalgae are known to be rich in omega-3. Hence, a more sustainable and high efficiency extraction method is required to ensure its viability. Sub-critical water extraction (SWE) is an emerging extraction technique as the technique involves shorter period of extraction, high efficiency and most importantly uses green and environmentally friendly solvent (water). In this preliminary experiment, different process conditions of SWE include temperature (156 – 274 °C), biomass loading (33 – 117 g/L) and retention time (6.6 – 23.4 minutes) were investigated on microalga, *Nannochloropsis gaditana*. The maximum yields of lipid and eicosapentaenoic acid (EPA) extracted were 17.86 wt.% of biomass and 15.78 wt.% of total fatty acid methyl ester (FAME), respectively. This productivity level (~16 wt.%) which is in keeping or higher than that of current production systems endorses SWE as a promising extraction technique for microalgal EPA production. Future works on optimization of SWE parameters will be performed to achieve the highest EPA concentration.

Keywords: eicosapentaenoic acid, green extraction, microalgae, omega-3, sub-critical

Abstrak

Mikroalga telah digunakan secara menyeluruh menghasilkan bahan api bio kerana ia mempunyai kadar kepekatan minyak yang tinggi. Bagaimanapun, tarikan semasa tertumpu pada penukaran minyak untuk menghasilkan produk berharga seperti omega-3. Banyak jenis mikroalga adalah kaya dengan omega-3. Oleh sebab itu, kaedah pengekstrakan yang mapan dan efisien diperlukan untuk menjamin daya maju mikroalga dalam penghasilan omega-3. Kaedah pengekstrakan air sub-kritikal (SWE) merupakan kaedah baru yang memerlukan masa pengekstrakan yang pendek, efisien dan menggunakan air sahaja sebagai pelarut. Dalam eksperimen awal ini, keadaan proses yang berbeza termasuk suhu (156 – 274 °C), muatan biojisim (33 – 117 g/L) dan tempoh pengekstrakan (6.6 – 23.4 minit) telah dikaji dengan menggunakan mikroalga, *Nannochloropsis gaditana*. Kadar maksimum minyak dan asid eikosapentaenoik (EPA) di ekstrak masing-masing ialah 17.86 wt.% daripada jumlah biojisim dan 15.78 wt.% daripada jumlah asid lemak metil ester (FAME). Kadar pengekstrakan yang tinggi ini (~16 wt.%) berbanding dengan kaedah semasa menunjukkan bahawa SWE ini ialah kaedah pengekstrakan yang mempunyai harapan untuk menghasilkan EPA daripada mikroalga pada masa hadapan. Pengoptimuman bagi parameter SWE untuk mendapatkan kadar kepekatan EPA yang paling tinggi akan dilakukan pada masa hadapan.

Kata kunci: asid eikosapentaenoik, pengekstrakan hijau, mikroalga, omega-3, sub-kritikal

Introduction

In the past years, people are becoming more health conscious due to the many health related problems arising in the community. People are in search of better quality oil to be consumed. Many studies related to polyunsaturated fatty acid (PUFA) especially omega-3 have been widely reported. Recent studies had proven more on the benefits of omega-3 towards the health implications of human. Long chain omega-3 had been known to have neuroprotective capabilities [1]. Meanwhile, omega-3 is also related to the reduction of risk in developing cardiovascular diseases. Some researchers found that the intake of 2 g/day to 4g/day of omega-3 fatty acid could reduce the triglyceride level in the blood [2, 3]. Other health benefits of omega-3 include anti-inflammatory potential [4, 5], reduction in the effect of tumour cells [6, 7] and aiding in Alzheimer's disease [8]. Some of the health and government organizations have even recommended a significant daily intake of omega-3 to reduce the chances of cardiovascular diseases [9]. Many had incorporated the omega-3 oil in fortifying food and also pharmaceutical products [10, 11]

Microalgae are considered to be one of the promising supplies of biomass for value add bio-products production due to their adaptability to thrive on limited land space and also high biomass productivity [12]. While microalgae have been extensively studied on the usage in biodiesel production [13], there are other bio-products that are worth studying and discovering through the extraction from microalgae. Current paper focuses on omega-3 from microalgae. Many microalgae species are high in omega-3 content [14]. Lately, big algae companies shifted their focus on obtaining omega-3 from algae sources and some even developed algal based omega-3 pharmaceutical products, showing a significant effect on the level of blood nutrient [15, 16]. With such valuable material, the extraction method could be an important indicator to improve the yield while reducing the usage of harmful material as well.

Previous studies had extracted EPA from microalgae using solvent extraction in comparison with other new techniques. Supercritical fluid extraction had been proven surpass the extraction efficiency of solvent extraction using acetone and hexane [17]. Meanwhile, sub-critical water extraction (SWE) of microalgae had been proven to be able to recover high amount polyunsaturated fatty acid from the cell [18]. Extraction using harmful organic solvent had no significant effect to improve the extraction yield but the solvent itself will actually harm the end product and also the environment [19]. SWE is one of the green methods to overcome the issues with solvent extraction method. This method mainly uses water as the solvent for extraction and the main principle is to change the properties of water under high temperature and pressure condition [20]. This method is known to be effective to extract products from microalgae such as *Nannochloropsis sp.* with high yield of lipid and omega-3 [21]. The strong cell wall of algae could be hydrolysed under this condition and the extraction of mainly non-polar content could be enhanced. Under sub-critical condition, this method is proven to allow shorter reaction time, lower operating cost and reduce or even omit the usage of harmful organic solvent [22]. Three parameters studied which are temperature, time and biomass loading are shown to have significant effect on the extraction yield [18].

Therefore, this article focuses on the preliminary study of the factors affecting the extraction yield which are extraction temperature, time and biomass loading on *Nannochloropsis gaditana*. This study is important towards the optimization of the process factors, targeting to improve the extraction yield while reducing the cost and time of the extraction.

Materials and Methods

Raw material and characterization

The 100% freeze dried biomass (*Nannochloropsis gaditana*) was purchased from Longevity Superfoods (Utah, USA). The powdered biomass was tightly sealed in the package and kept in dry condition to maintain the quality of the microalgae. The carbon, hydrogen, nitrogen and sulphur (CHNS) analysis were done in a CHNS628 analyzer. Ultimate analysis is important to characterize the biomass because each batch of cultivated biomass will be different. Soxhlet extraction method with hexane as the solvent was used to determine fat content of the biomass. Protein content was determined using the Kjeldahl method [23]. Moisture content was determined by the differences mass before and after the drying process in the oven at 105 °C followed by the incineration step at 550 °C to determine the ash content. Carbohydrate content was taken as the sum of residual after all other contents were determined.

Extraction equipment and procedures

The main components of the system consist of a salt bath, temperature controller unit and a stainless steel reactor (Swagelok 316 SS; 3/4 in. OD x 0.049 in. wall). The stainless steel reactor was filled with the desired amount of biomass (microalgae) and water (33 – 117 g algae/L). The tightly screwed reactor then placed into the salt bath and run with the desired temperature (156.1 – 273.9 °C) and extraction time (6.6 – 23.4 minutes). All the parameters chosen were based on the range of parameters used by similar studies [18].

The reacted biomass in SWE was centrifuged at 4000 rpm for 10 minutes. The supernatant consists of oil layer was decanted by adding 10 mL of hexane and the process was repeated for 3-4 times to ensure the oil layer was completely recovered. The hexane and crude lipid mixture were then transferred to a rotary evaporator to recover the excess hexane. Meanwhile, the weight of the lipid was determined. The oil samples were tightly sealed in containers and kept in the freezer no longer than 6 months for further analysis.

Gas chromatography analysis

The dried crude lipid sample was mixed with 1 mL of hexane. 1 mL of sodium methoxide was added to the mixture of hexane and oil for transesterification reaction to take place. The mixture was then stirred for 10 seconds on a vortex stirrer, and the top layer of fatty acid methyl ester (FAME) was decanted for GC analysis.

The GC analysis was done on Hewlett-Packard 5890 Series II Plus GC with an automatic liquid sampler and flame ionization detector (FID). The stationary phase chosen was (50%-Cyanopropylphenyl)-dimethylpolysiloxane and the column used in this analysis was DB-225 (30 m × 0.25 mm × 0.25 µm). Splitless mode was used injecting 1.0 µL of sample to be analyzed. The carrier gas used was hydrogen with a set flowrate of 1.3 mL/min. Initial oven temperature was preset to 35 °C with holding time of 0.5 minute. Then the temperature was ramped up to 195 °C with heating rate of 25.0 °C/min. Then temperature was raised at 3.0 °C/min to 205 °C and then at 8.0 °C/min to 230 °C and held for 6.7 minutes. The total run time was 20.06 minutes. The detector temperature was set to 240 °C with nitrogen gas as make-up gas. The standard used for the analysis to compare all the peaks showing in the sample was Supelco® 37 Component FAME Mix standard.

Results and Discussion

Ultimate and proximate analysis

The biomass was characterized *via* CHNS and proximate analysis. The percentage of carbon, hydrogen, nitrogen and sulfur in the biomass were 47.91%, 6.83%, 7.56% and 0.63%, respectively. With the high carbon percentage, this microalgae biomass possesses a significant potential for biofuels and bio-products production. Meanwhile, potential products from the biomass can be developed from the proximate analysis in Table 1.

Table 1. Proximate analysis of *Nannochloropsis gaditana*

Content	Weight percent (wt.%)
Fat	10.2
Moisture	2.9
Ash	10.8
Carbohydrate	28.9
Crude Protein	47.2

High percentage of fat obtained from Soxhlet extraction showed that *Nannochloropsis gaditana* has potential to be used as omega-3 oil source. Also, the presence of carbohydrates and protein compositions in the biomass has potential for other bio-products (e.g. chemicals and feed) production.

Maximum lipid and EPA yield

The oil yield was calculated based on the final oil yield (g) extracted compared to the initial mass loading (g) of the biomass to the reactor. The maximum oil yield from both Soxhlet and SWE showed a significant difference as shown in Figure 1. It was found that oil yield of 10.2 wt.% was produced *via* Soxhlet extraction method whereas the SWE method produced higher oil yield of 17.86 wt.% using temperature at 215 °C for 15 minutes with 75 g algae/L as compared to Soxhlet extraction.

From GC analysis, the results showed a positive finding on the extraction of EPA from *Nannochloropsis gaditana*. The EPA compositions yielded the highest amount of up to 15.78 wt.% of total FAME using temperature at 215 °C for 6.6 minutes, with 75 g algae/L biomass loading. Meanwhile, the Soxhlet extraction yielded of 28.10 wt.% of total FAME. The lower amount of EPA yield obtained using SWE as compared to Soxhlet might be due to the degradation of some EPA during the extraction process as the process was not optimized [18]. Meanwhile, the Soxhlet extraction with a lower oil yield showed that oil was not fully recovered even though there might be a higher percentage of EPA in the oil. The oil yield is important as the lipids in microalgae mostly exist in the natural form with all the fatty acids attached to mainly glycerol, carbohydrates and phosphates [24]. Hence, high oil yield might increase the amount of EPA in the oil. These results proved that SWE is a safer and environmentally friendly process that can be used as an alternative method for EPA extraction in the future with further optimization.

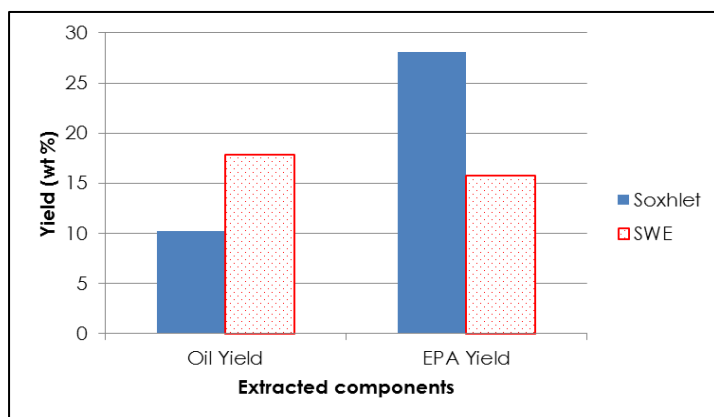


Figure 1. The maximum extraction yield for both Soxhlet and SWE

Effect of Temperature

Temperature is one of the significant parameters when dealing with SWE [20]. The findings on the effect of temperature for *Nannochloropsis gaditana* extraction using SWE are shown in Figure 2. The study found that the highest EPA (14.14 wt.% of total FAME) and oil (17.86 wt.% of biomass) yields were obtained at 215.0 °C.

The temperature setting affects the yield of the oil as the condition of the sub-critical water changes. As the water is heated up to a higher range of temperature, the water hydrolysis power will be higher [22]. Hydrolysis reaction is good in breaking up the cell wall of the microalgae. However, when the reaction is high in hydrolysis, degradation of oil occurs, hence, decreasing the oil yield as well as quality at high temperature. Meanwhile, the increase in temperature under SWE condition also increases the solubility of water to extract non-polar substrates such as triglycerides which holds the omega-3 [20].

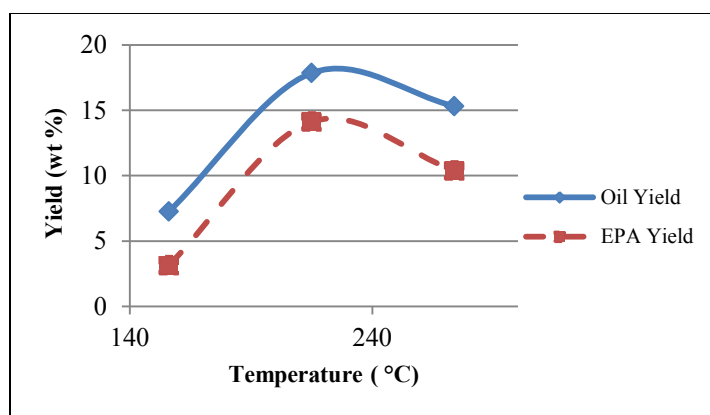


Figure 2. The effect of temperature on oil and EPA yield

Effect of extraction time

The extraction time is also one of the important factors to be considered when performing SWE. Although reducing the time of extraction might reduce the extraction cost, however, increasing the time of extraction could improve the yield of the bio-products as shown in Figure 3.

Figure 3 shows the effect of extraction time on the oil and EPA yields. The effect of reaction time affects the oil yield. From Figure 3, shorter reaction time is advantageous as it improves the EPA extraction to have lower denaturation effect. However, the shorter time of extraction does not allow a complete extraction process and leaving out most of the lipid in the microalgae. Meanwhile, at longer extraction time, due to the EPA is exposed under high temperature, the EPA will be denatured and there might be possibilities of secondary or tertiary reaction happening under SWE condition [25].

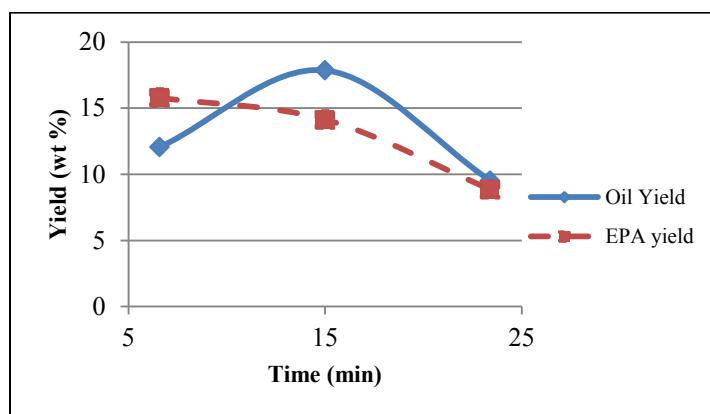


Figure 3. The effect of extraction time on oil and EPA yield

Effect of biomass loading

Dewatering and drying of microalgae involve high operational cost in the current microalgae harvesting industries [26]. Hence, low biomass loading or high water content extraction process would be favourable in reducing the initial cost of harvesting. Figure 4 shows the effect of biomass loading on oil and EPA yields.

From Figure 4, it was found that low biomass loading favoured the extraction of oil from the biomass. Lower biomass loading means more water could be used to extract the total lipid content in the biomass. However, the total

EPA was reduced at low biomass content. This might be due to the secondary or tertiary reaction happened at high water content, degrading the lipid quality in terms of omega-3 extraction. The reduction of EPA yield as compared to other FAME also showed that EPA is highly susceptible to oxidation due to its long chain of carbon as compared to other FAME [27].

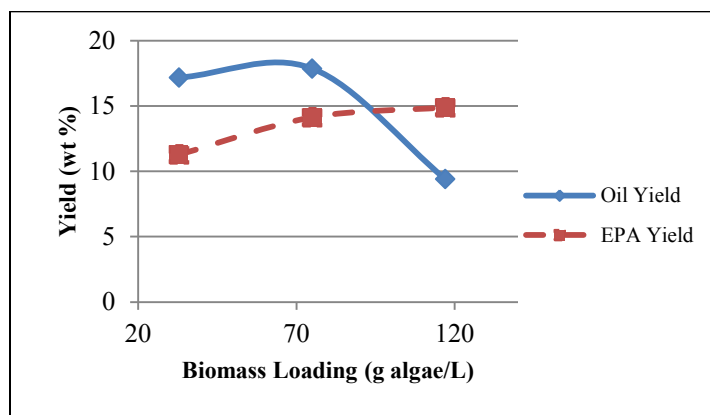


Figure 4. The effect of biomass loading on oil and EPA yield

Conclusion

This study discusses the preliminary results of using SWE to extract omega-3 from microalgae, *Nannochloropsis gaditana*. Utilizing SWE and microalgae as the source of omega-3 production are proven to be a sustainable and environmentally friendly as compared to harmful organic solvent usage. While the extraction yield of EPA might not be as high as conventional solvent extraction, this extraction process showed a significant extraction capability with the significantly high oil yield from SWE (17.86 wt.%). Hence, this study hopes to be a precursor of further optimization study on the extraction of omega-3 using SWE to improve the extraction process while making SWE possible to be scaled-up.

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