UNIVERSITI PUTRA MALAYSIA

EXTRACTION OF LIPIDS AND EICOSAPENTAENOIC ACID FROM NANNOCHLOROPSIS OCEANICA BIOMASS USING IONIC LIQUID-BASED MICROWAVE-ASSISTED EXTRACTION METHOD

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SHIVA REZAEI MOTLAGH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

December 2020

DEDICATION

This study is wholeheartedly dedicated to my beloved parents, who have been my source of inspiration and gave me strength when I thought of giving up, who continually provide their moral, spiritual, emotional, and financial support.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EXTRACTION OF LIPIDS AND *EICOSAPENTAENOIC ACID* FROM *NANNOCHLOROPSIS OCEANICA* BIOMASS USING IONIC LIQUID-BASED MICROWAVE-ASSISTED EXTRACTION METHOD

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December 2020

Chairman: Assoc. Prof. Razif Harun, PhD
Faculty: Engineering

The consumption of omega-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA, 20:5n-3), have some therapeutic impacts on human health. The combination of ionic liquids (ILs) with MAE revealed significant attention in comparison to conventional toxic solvents for biomass lipid extraction. Therefore, this study analyzes the suitable ILs for extracting EPA from microalgae, *Nannochloropsis oceanica*. The scope of the study includes screening of ILs solvents to be used using computational COSMO-RS, extracting the lipids from the microalgae, evaluating the kinetics and the thermodynamics of the extraction process as well as manipulating the extraction process for optimum EPA yield.

It was found that among the investigated ILs, a combination of tetramethyl ammonium with SO_4 or Cl was the best fit for EPA extraction. Three selected ILs, namely [EMIM][Cl], [TMAm][Cl], and [EMPyrro][Br], were empirically selected based on results provided by COSMO-RS software. The validation results pinpointed the good prediction capabilities of COSMO-RS.

Furthermore, the study investigates the effect of different extraction parameters of MAE with ILs including solid-loading, irradiation time, irradiation power, extraction temperature, and IL amount on the total lipid extraction yield and EPA content from microalga *Nannochloropsis oceanica*. The results of the screening study shown that the highest amount of total lipid yield and EPA content for all examined ILs were extracted with examined ILs mediated in water-based microwave irradiation at the ILs amount of 2 g, 25 min, 90 °C and 1 g of ILs amount, 25 min, 90 °C, respectively.

The optimum extraction conditions, the yield of total extracted lipids and the content of EPA were found 19.58 wt% (gr/gr of total biomass) and 37.919 mg/g of total biomass respectively, when the extraction process was accomplished in 24.69 min at 88.18 °C and with 1.65 g of [TMAm][Cl]. Furthermore, at similar condition, the total lipid and EPA were extracted in the order of: [TMAm][Cl] > [EMPyrro][Br] > [EMIM][Cl].

Under these experimental conditions, total SFAs, MUFAs and PUFAs composition after ILs based MAE were respectively 28.49 wt%, 27.99 wt% and 40.63 wt% for [TMAm][Cl], which the high percentage of PUFAs make it a potential feedstock for pharmaceutical production. Also, [TMAm][Cl] mediated in water medium based microwave irradiation had improved the total lipid yield and EPA content around 2.53 times and 7.85 times at the optimum condition in comparison to the standard extraction method of Soxhlet which was extracted total lipid around 7.75% and 4.83 mg/g of EPA.

Among the mathematical models used, Second-order rate law and Patricelli model with highest R^2 of 0.9823 and 0.968 were recognized as the most suitable model for explanation of extraction processes of [TMAm][Cl] based microwave irradiation of EPA extraction.

The values of thermodynamic parameters such as Gibbs free energy change (ΔG), enthalpy change (ΔH) of +403.0557 (kJ/mol.K), and entropy change (ΔS) of + 0.1485 (kJ/mol.K) indicate that this process is endothermic, irreversible, and spontaneous, respectively. Overall, the IL-MAE method promises a cheaper, greener, and faster extraction process for a high-quality lipid and EPA from microalgae.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGEKSTRAKAN LIPID DAN ASID EICOSAPENTAENOIC DARI NANNOCHLOROPSIS OCEANICA BIOJISIM MENGGUNAKAN KAEDAH KETUHAR GLOMBANG MIKRO BERASASKAN CECAIR IONIC

Oleh

SHIVA REZAEI MOTLAGH

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Pengambilan omega-3 asid lemak tidak tepu (PUFA), seperti acid eicosapentaenoic (EPA) dapat memberikan kesan terapeutik terhadap kesihatan manusia. Penggunaan cecair ionik (IL) dengan ketuhar sinaran gelombang mikro (MAE) mendapat perhatian yang signifikan berbanding dengan pengunaan pelarut toksik dalam mengekstrak lipid dari mikroalga biojisim. Oleh kerana itu, kajian ini menganalisis IL yang sesuai untuk digunakan dalam proses pengekstrakan EPA dari mikroalga *Nannochloropsis oceanica*. Skop kajian ini merangkumi pengunaan perisian komputer COSMO-RS dalam mengenalpasti pelarut IL yang sesuai digunakan untuk mengekstrak lipid dari mikroalga, menganalisa kinetik dan termodinamik untuk proses pengekstrakan serta memanipulasi proses pengekstrakan untuk mendapatkan hasil EPA yang optimum.

Hasil kajian menunjukkan di antara IL yang diselidiki, gabungan di antara tetramethyl ammonium dengan SO_4 atau Cl adalah gabungan yang paling sesuai untuk mengekstrak EPA. Bedasarkan hasil penemuan daripada perisian COSMO-RS, tiga IL, iaitu [EMIM][Cl], [TMAm][Cl], dan [EMPyrro][Br], telah dipilih secara empirik. Hasil pengesahan juga menunjukkan kemampuan ramalan COSMO-RS yang baik. Hasil daripada kajian saringan menunjukkan bahawa hasil tertinggi lipid dan kandungan EPA untuk semua IL yang diselidiki adalah diekstrak dengan IL yang yang dimediasi dalam penyinaran gelombang mikro berasaskan air dengan menggunakan IL 2 g, 25 min, 90 ° C untuk lipid dan 1 g ILs, 25 min, 90 ° C untuk EPA.

Pada kondisi yang optimum, jumlah keseluruhan lipid dan EPA yang diekstrak ialah 19.58 wt% (gr/gr jumlah biojisim) dan 37.919 mg/g FAME. Proses pengekstrakan

ini mengambil masa 24.69 min pada suhu 88.18 °C dengan menggunakan 1.65 g [TMAm][Cl]. Selanjutnya, pada keadaan yang sama, jumlah lipid dan EPA diekstrak mengikut urutan IL ialah: [TMAm][Cl]> [EMPyrro][Br]> [EMIM][Cl].

Di bawah keadaan eksperimen ini, jumlah komposisi asid lemak tepu (SFA), asid lemak tidak tepu mono (MUFA) dan asid lemak tidak tepu poli (PUFA) setelah MAE menggunakan IL masing-masing adalah 28.49 wt%, 27.99 wt% dan 40.63 wt% untuk [TMAm][Cl]. Peratusan tinggi PUFA yang dijumpai dalam kajian ini menjadikannya bahan makanan berpotensi untuk produk farmasi.

Jumlah hasil lipid dan kandungan EPA yang diekstrak dengan menggunakan [TMAm][Cl] yang dimediasi dalam penyinaran gelombang mikro berasaskan medium air pada keadaan optimum adalah 2.53 kali dan 7.85 kali lebih banyak berbanding dengan kaedah pengekstrakan standard Soxhlet yang cuma mengekstrak lipid sekitar 7.75% dan 4.83 mg/g EPA.

Di antara model matematik yang dikaji, model kinetic kedua dan model Patricelli diiktiraf sebagai model yang paling sesuai untuk menerangkan mengenai proses pengekstrakan menggunakan sinaran gelombang mikro berdasarkan [TMAm][Cl]. dengan nilai R^2 0.9823 dan 0.968.

Nilai parameter termodinamik seperti perubahan tenaga bebas Gibbs (ΔG), perubahan entalpi (ΔH) +403.0557 (kJ/mol.K), dan perubahan entropi (ΔS) + 0.1485 (kJ/mol.K) menunjukkan bahawa proses pengekstrakan ini bersifat endotermik, tidak boleh kembali pada asal dan spontan. Secara keseluruhan, kaedah IL-MAE menjanjikan proses pengekstrakan yang lebih murah, lebih hijau dan lebih pantas untuk lipid dan EPA yang berkualiti tinggi dari mikroalga.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

NLs	Neutral lipids
FAs	Fatty acids
PLs	Phospholipids
AGs	Acylglycerols
GLs	Glycolipids
MAG	Monoacylglycerol
DAG	Diacylglycerol
TAG	Triacylglycerol
FFAs	Free fatty acids
LD	Lipid droplet
N	Nucleus
ST	Stacks of thylakoids
MAE	Microwave-assisted extraction
MW	Microwave
UAE	Ultrasound Assisted Extraction
SWE	Subcritical water extraction
SFE	Supercritical fluid extraction
PSE	Pressurized solvent extraction
EAE	Enzyme-assisted extraction
ILs	Ionic liquids
ASE	Accelerated Solvent Extraction
B&D	Bligh & Dyer

LIST OF NOMENCLATURES

ε'	Dielectric constant
ε''	Loss factor
P_{diss}	Microwave power dissipation per unit volume
F	Frequency
E	Electric field strength
N_A	Mass flux of target solute (A) across the liquid film (mg solute A, m^{-2}/min)
k_L	Liquid film mass transfer coefficient (m/min)
C_{AL}^{*}	Concentration in the liquid phase at equilibrium (mg $A \cdot m^{-3}$)
C_{AL}	Concentration of solute A in the bulk liquid (mg $A \cdot m^{-3}$)
$\frac{dC_{LA}}{dt}$	Extraction rate of solute A into the bulk liquid phase (mg solute A m^{-3}/min)
A_s	Surface area (m^2)
V	Volume of solution (m^3)
T	Extraction time (min)
α	Conversion factor
m_s	Amount of solid sample (g)
k	Overall volumetric mass transfer coefficient
a_s	Interfacial surface area (m^{-1})
C_{eq}	Concentration of solute A at equilibrium and solid–liquid interface (mg solute A/g.dw solid material)
C_t	Concentration of solute A in the bulk liquid phase (mg solute A/g.dw solid material)
C^w	Final oil concentration in the solvent phase for the washing stage
C^d	Final oil concentration in the solvent phase for the diffusion stage
k^w	Kinetic coefficient for the washing stage
k^d	Kinetic coefficient for the diffusion stage
C_2^d	is the final yield (hypothetical) in solution due to the second diffusion stage alone
k_2^d	Kinetic coefficient for the second diffusion stage
C_e	Final concentration of products for washing and both diffusion stage at equilibrium state
k_1	First-order extraction rate constant ($l.g^{-1}.min^{-1}$)
C	Extraction capacity of the target compound
C_o	Initial concentration of the target compound
A_1	Pre-exponential faction
k_2	Second-order extraction rate constant ($l.g^{-1}.min^{-1}$)
C_s	Extraction capacity (concentration of target compound at saturation in $g.l^{-1}$)
h	Initial extraction rate
β	Extraction rate constant ($l.g^{-1}$)
DOE	Design of Experiment

OFAT	One factor at a time
RSM	Response surface methodology
CCD	Central composite design
ANOVA	Analysis of variance
COSMO-RS	Conductor like screening model for realistic solvents
$CHCl_3$	Chloroform
MeOH	Methanol

CHAPTER 1

INTRODUCTION

1.1 Research Background

The regular consumption of omega-3 polyunsaturated fatty acids (PUFAs) and predominantly in its form of eicosapentaenoic acid (EPA, 20:5n-3) is crucial for human body as it is proved to significantly mitigate the risk of heart diseases, neurotic disorders, asthma, depression and inflammation (Shahidi & Ambigaipalan, 2018; Rezaei Motlagh et al., 2019; Arita, 2016; Chowdhury et al., 2012; Fabian, et al., 2015). Fish is considered as the most abundant source of these PUFAs. However, the intake of fish oil for months may cause a deficiency of vitamin E due to the high level of vitamin A and D in fish lipids (Lenihan-Geels et al., 2013). The presence of many harmful contaminants such as methyl mercury, copper and organic pollutants as polychlorinated biphenyls (PCBs) or dioxins are also found in some species of fish especially in salmon, sardine, anchovy, tuna which may impose toxic effect to human health (Ryckebosch et al., 2012). Furthermore, the availability of fish is seasonal, which may hamper the continuity of food (Handayania & Ariyantib, 2012). Therefore an alternative source of omega-3 PUFAs is required to overcome the issues. Fish do not produce omega-3; they will get it by eating microalgae. This realization has turned microalgae into one of the most important producers of omega-3 PUFA including ALA (C18:3), EPA (C20:5) and DHA (C22:6) (Adarme-vega et al., 2012; Ryckebosch et al., 2012). When comparing to fishery and associated food industries, microalgae cultivation offers some undeniable advantages to be used as a sustainable source of omega-3 PUFA with the ability to have a very high growth rate and low environmental impacts. They are not only growing in briny, saline, and coastline seawater with little competition, but wastewater also serves as their habitant to provide the nutrients necessary for their survival.

Furthermore, environmental advantages can be obtained as they consume carbon dioxide from effluent gases (Fajardo et al., 2007; Pragya et al., 2013). Thus, microalgae, especially marine microalgae can be considered as crucial renewable source of omega-3 PUFA. In spite of the current high cost of microalgae production, this feedstock is viewed as a great attractive source due to the potential of high oil yields (up to 100,000 L of oil/ha/year) which is about 16–70 times the oil yield/ha/year achieved from palm, coconut, castor, and sunflower (Demirbas & Demirbas, 2011). Some species of microalgae have high oil content (exceeding 80% of their dry mass (Satyanarayana et al., 2011)) and it is desirable to extract this oil effectively in a cost efficient manner. Figure 1.1 shows a comparison of six *Nannochloropsis sp.* microalgae types in terms of lipid content (Radakovits et al., 2012). Among the various types of microalgae, *Nannochloropsis oceanica* has the highest quantity of lipid ($mg.l^{-1}.d^{-1}$).

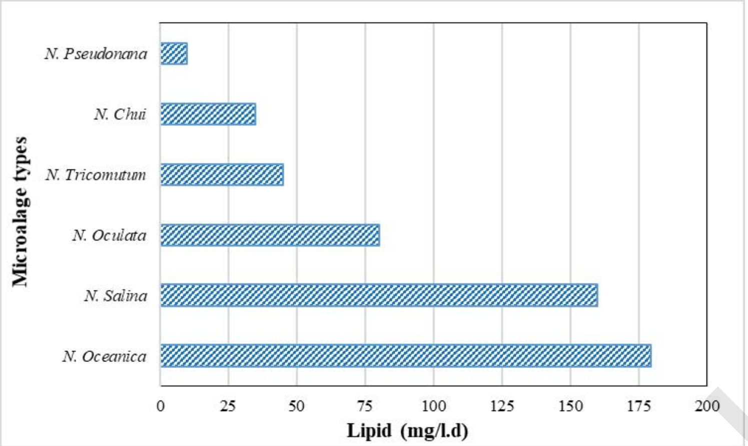

Figure 1.1: Comparison of *Nannocloropsis sp.* microalgae types in lipid production rate (Radakovits et al., 2012)

Microalgal lipids are trapped inside the cell wall. A suitable extraction method is the most crucial and vital process for the production of high microalgal lipids. However, microalgal cells are challenging to disrupt due to the complex polymers presence within the cell walls such as algaenan and sporopollenin. Solvents and disruption devices are required to extract the target compounds out from the cells. For many years, the conventional method of Soxhlet extraction using hexane has been broadly used for lipid extraction from microalgal biomass however, a few weaknesses has been attributed to this method and mostly associated with its commercial viability. Typically, the walls of microalgae cells are comprised of a complex structure of polysaccharides intercalated with proteins which makes the microalgae chemically stable against the nonpolar solvents (Mubarak et al., 2015). When in form of lipid droplets, the hexane as solvent, cannot act efficiently in breaking down the protein bound, crossing through the polar phospholipid-membrane and adequately extracting the lipids. To resolve this problem, the popular Bligh & Dyer method is often used as an alternative in which the hexane is replaced by a polar solvent such as methanol and chloroform. The polar solvent can diffuse into the phospholipid matrix of the cell walls and therefore, facilitate the extraction of lipid. Both methods of Soxhlet and Bligh & Dyer, despite being conventionally used, are, however, are susceptible to be expensive, limited by low efficiencies and un-ecofriendly.

Furthermore, microwave assisted extraction (MAE) shows a promising technique for extracting biochemical components particularly microalgal lipids and omega-3. It has many advantageous include reducing extraction times and improving extraction yields and purity of products (Chemat & Cravotto, 2012). MAE is the process by which microwave energy is used to heat solvents in contact with solid samples and to partition compounds of interest from the sample into the solvent (Flores, 2014). MAE can reduce both extraction time and solvent consumption as compared to the conventional methods (Pan et al., 2016). Also, this method has improved the extraction rate of a variety of compounds from biomass (Azmir et al., 2013). MAE, via applying the prompt heating and imposing pressure to the biological substance, facilitates the transfer of target compounds from the polysaccharide matrix of microalgae biomass into the solution medium hence provides a high-quality extracts with efficient stage of recovery (Singh et al., 2017). In MAE, through rapid increase of temperature, localized hot spot and regular pressure gradient will appear in a microalgae cell leading to cellular wall degradation thus increases the total rate of mass transfer (Kanitkar et al., 2011). MAE may extract lipid with higher proficiencies when utilizing solvents rather than applying mechanical oil expulsion techniques. Additionally, both polar and non-polar solvents can be employed for extracting oils and other essential components from biological materials. The choice of solvent (polar or non-polar) relies on the polarity of the target compounds. It delivers mutual solubility; polar compounds are better extracted using polar solvents and vice versa. For extracting the vegetable oils, hexane has been widely utilized because of its inexpensive and boiling point, despite of its dangerous nature (Routray & Orsat, 2012).

1.2 Problem Statement

Most organic solvents require effective recovery process for commercialization and contribute to air pollution problems. This is due to their relatively high volatilities, as well as they are vulnerable for being toxic, flammable, and relatively expensive and sometimes for some particular compounds they may not work well due to their low solubilizing ability. In the case of microalgae, lipids are typically trapped inside the cell walls, comprising proteins, large and complicated polysaccharide compounds that make them high chemically resistant to no-polar solvents (Le Costaouëc et al., 2017). To extract the lipids while in the form of a droplet, hexane is needed to cross the polar phospholipid-membrane (*i.e.* the protein band) which seems hard to achieve. Nevertheless, polar/non-polar solvents such as methanol and chloroform, which are regularly applied in Bligh and Dyer method, are cable of crossing the phospholipid membrane through diffusion and henceforth extract the lipids a result (Halim et al., 2012). Therefore, these conventional methods are either time, solvent-consuming, and too environmentally unfriendly (Mercer & Armenta, 2011; Ranjith Kumar et al., 2015).

In recent times, ionic liquids (ILs) have been attained substantial attention in comparison with the conventional toxic solvents *e.g.,* hexane, chloroform and methanol for biomass lipid extraction (Choi et al., 2019; Chua et al., 2018; Kodolikar Kulkarni et al., 2018; Kumar et al., 2018; Wahidin et al., 2018). The application of ILs as solvent extraction offers a number of advantages, such as remarkably negligible vapor pressure, excellent solubility and high thermal stability, making them appropriate for containment, recycling and regeneration of products (Tan et al., 2012). The stability of ILs includes thermal, electrochemical, radiolytic, and chemical types. Almost all the utilization of ILs relates to a relatively high temperature, such as desorption, catalysis, and solvents. One of the most important reasons for the boom of ILs lies on their high thermal stability, *i.e.*, innerness to decomposition and evaporation. Thermal stability is the precondition for the widespread industrial application of ILs. Additionally, ILs require lower energy than organic solvents due to their special features to help the extraction by reducing the complexity of the process (Bahadur et al., 2016). Furthermore, ILs were recognized as valuable tools for lignocellulose pretreatment since they could disturb the hydrogen bonds and expose the lignocellulosic material to the cellulose which improves the extraction process (Yang et al., 2013). High efficiency of lipid extraction utilizing ILs based MAE is due to the high polarity and high dielectric properties of ILs make it an efficient solvent under microwave irradiation. In which the microwave transforms electromagnetic energy into heat based on two mechanisms: dipole rotation and ionic conduction. The polar compounds realigning in the direction of electric field (Pan et al., 2016). So, most of the ILs could reduce the worries regarding toxicity and environmental issues in comparison to conventional organic solvents. Therefore, this study assesses the suitable ILs to be used in the extraction process of EPA from microalgae *Nannochloropsis oceanica*. The scopes of the study includes the screening of suitable ILs solvents to be used using computational COSMO-RS, evaluating the kinetics, and the thermodynamics of the extraction process as well as manipulating the process for optimized total lipid yield and EPA content.

1.3 Objectives

The main aim of the study is to assess the feasibility of ILs based microwave-assisted extraction as the green extraction technology approach for lipid and eicosapentaenoic acid (EPA) from *Nannochloropsis oceanica* biomass. The specific objectives for this study include:

1. To select suitable ILs with high capacity values for the extraction of eicosapentaenoic acid (EPA) from *Nannochloropsis oceanica* by solvent screening via computational COSMO-RS.

2. To optimize the microwave-assisted extraction of lipid and EPA from *Nannochloropsis oceanica* using ILs.

3. To evaluate the kinetics and thermodynamics properties of the extraction process of EPA from microalgae *Nannochloropsis oceanica* via IL based microwave irradiation.

1.4 Scope of the Study

1.4.1 Selection of suitable ILs via computational COSMO-RS

A screening evaluation using the COSMO-RS method to determine the capacity values at infinite dilution of five different types of cation with 22 anions based ILs (352 cations–anions combination) in order to identify the most suitable types to apply to solid–liquid extraction of EPA from microalgae. Then, the predicted extraction capacity of EPA for selected ILs from COSMO-RS were validated by the experimental part.

1.4.2 Screening the process parameters

One factor at the time (OFAT) was performed to investigate the influence of main operation parameters on the extraction of crude lipid and EPA quantity by studied the effect of H_2O volume (5 to 50 ml), extraction time (5 to 50 min), irradiation power (300 to 800 watt), temperature (50 to 100 °C) and IL amount (0-3.5 g). Then, the effect of ILs amount on the extracted total lipid, EPA content and fatty acids profile were investigated by selected ILs from COSMO-RS study namely [TMAm][Cl] and [EMPyrro][Br] with [EMIM][Cl].

1.4.3 Investigation and optimization of process parameters

The application of Response surface methodology (RSM) was employed to evaluate the interaction of parameters with the method of central composite design (CCD). The selected independent factors were reaction temperature (60 to 100 °C), reaction time (5 to 30 min) and IL amount (0.5-2.5 g) and the respected responses were the total lipid yield (% wt. of total biomass) and content of EPA (mg/g of total biomass). An optimization method of "The desirability approach" was used to identify the optimum operating conditions. The extraction yield of crude lipid and content of EPA using ILs based MAE method was compared with the conventional method of Soxhlet extraction. The selected ILs from COSMO-RS study ([EMIM][Cl], [TMAm][Cl] and [EMPyrr][Br]) was compared at the optimum condition from CCD study.

To support the experimental study, FTIR analysis was conducted on the solid residual of samples after Soxhlet and [TMAm][Cl] based microwave irradiation at the optimum condition and compare with the raw microalgae.

1.4.4 Evaluation of kinetics and thermodynamics properties

The extraction kinetics of MAE of EPA content from microalgae *Nannochloropsis oceanica* under the influence of temperature were examined by demonstrating the extraction curves (yield vs. time) follows First-order rate law, Second-order rate law and Patricelli models. The kinetic study conducted using [TMAm][Cl] ILs which selected from screening part using COSMO-RS.

The thermodynamic parameters of extracted EPA content from microalgae *Nannochloropsis oceanica* under the influence of temperature and time was investigated. The thermodynamic analysis is assessed to obtain the enthalpy, entropy, and Gibbs free energy of the reaction.

1.5 Significant of Research

Firstly, it was found that none of the published article reported on the use of COSMO-RS as a prediction tool to screen the appropriate ILs for the extraction of

EPA compounds. Therefore, this study's innovation is the use of COSMO-RS for the prediction purpose as a part of solid-liquid extraction. Henceforth, this study is significant not only for a researcher who works on EPA extraction but also it is a new horizon for researchers seeking the extraction of short-chain and other long chain fatty acids using ILs.

Secondly, this study focuses on developing the microwave irradiation technology for enhancing the quantity and quality of lipid extraction and EPA from *Nannochloropsis oceanica* microalgae using ILs by decreasing the overall energy consumption and duration time.

The most important target of this study is that to overcome the limitation of ILs regarding their relatively high price compared to other solvents, the lipid and EPA were extracted on the IL in a water medium. It is not only beneficial for reducing the IL amount but also to enhance the extraction efficiency of lipid and EPA in a short period and lower temperature.
There is no literature available on the use of ILs in water medium with the aid of microwave irradiation to extract lipid and EPA quantity. It is due to most of the literature focus on the use of ILs and microwave to the production of total lipids for biofuels. Moreover, the microalgae *Nannochloropsis oceanica* was chosen in this study since it is known as EPA-rich species with thick cell walls. The extraction of EPA quantitative data have not yet been described for this species.

Furthermore, EPA extraction has numerous profits in both pharmaceutical and nutraceutical industries. The lipid and EPA obtained could alleviate the shortage of EPA and omega-3 in the future, therefore confirmed the ability of microalgae as one of the new lipid source and microwave irradiation as a feasible and environmentally friendly system. The fatty acid profiling in this study could categorize the type of fatty acids that are appropriate to be produced via the MAE process. The optimization study could provide an optimum operating condition for high yield lipids and EPA production. Last but not least, the result of the kinetic study is valuable in helping us realize the mechanisms of total lipid and EPA production from the microwave irradiation process by investigating the release of the microalgae cell contents into the extraction solvent.

1.6 Structure of dissertation

The thesis comprise of five chapters following the structures. Chapter One introduced the background about significance of EPA and the scope of current research, furthermore emphasized on the objectives taken to overcome the research problem. Chapter Two discusses the literature surrounding microalgae lipid extraction and ionic liquids. The chapter additionally studies the extraction methods applied in microalgae extraction particularly the MAE technique. Topics covered in MAE involve the development of the technique in plant extraction, influencing parameters of MAE, advantages and disadvantages related to other extraction techniques and the kinetic study of lipid extraction was reviewed. Research methodology has been comprehensively described in chapter Three. The final results have been presented and discussed in detail in chapter Four. Chapter Five is the final conclusions to highlight the most noteworthy aspects and outcomes from the study and recommends a new research area for future studies.

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