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Improving Recovery Process of Omega-3 Fatty Acids from a Native Species of *Chlorella vulgaris* Using Integrated Method

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Abstract

Background and Objective: Fish tissue and liver and some microalgae are some of the richest sources of omega-3 (ω3) fatty acids in nature. Annually, more than 4.2 billion tons of algae are cultivated with various goals. Therefore, development of novel ways to extract more ω3-fatty acids from microalgal tissues is a valuable target. In this study, comparisons were carried out between various methods of extraction and purification of ω3-fatty acids from a native species of microalgae.

Material and Methods: Combination method (urea complex and winterization) used as a method for extraction of ω3-fatty acids. For the optimization of combination method, three factors, including temperature of winterization, time of winterization and frequency of extraction with hexane were studied at four various levels and 15 examinations using Design Expert 10.0 software.

Results and Conclusion: Results of optimization included 10 h for time of winterization, -20 °C for temperature of winterization and six times of extraction with hexane. In optimized conditions, the highest quantity of ω3-fatty acids derived from *Chlorella vulgaris* at the moisture of 65% was 43.6 mg g^{-1} fatty acids.

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1. Introduction

In several studies from 1988, various methods such as partial distillation, high performance liquid chromatography (HPLC), separation with supercritical fluid and separation with urea complex for the extraction and purification of fatty acids (FAs) from body tissues, fish livers and microalgae have been suggested. The most important role of fats in the body is their presence in cell membranes. Energy of 1 g of fats is nearly 19 kcal, which is twice as much as that of sugars and hydrocarbons. The FAs are divided to two major categories; unsaturated FAs (UFAs) and saturated FAs (SFAs). Human body does not produce some of these FAs, which are called essential FAs (EFAs) [1]. The most important EFAs that the body cannot produce include omega-3 (ω3) FAs. The ω3-FAs are divided into three major categories of alpha-linolenic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [2]. The alpha-linolenic acid type of ω3-FAs is present in walnuts, flax-seeds, olives and soybeans. The other two types of ω3-FAs are exclusively present in seafood [3]. The ω3-FA market size was USD 4.1 billion in 2019 and is projected to grow at an annual growth rate of 13.1% to reach a value of USD 8.5 billion by 2025. Today, the major source of ω3-FA production is fish oil, which contains 30% of ω3-FAs. In fact, less than 100,000 tons of ω3-FAs per year can be achieved from fish oils. Accordingly, it has been predicted that fish oil alone is not able to supply the market demand. Therefore, use of novel ω3-FA sources such as FAs from microalgae seems to be necessary and commercializable [4,5]. Using closed culture systems and providing necessary elements to microalgae, it is possible to produce biomasses with no contaminations at favorite quantities. Using one of ω3-FA purification methods, it is possible to achieve opti-

mal and economical production of ω-FAs from micro-algae [6,7]. There are several ways to recover ω3-FAs from microalgae. Fractional crystallization at low temperatures is one of the simplest methods used to produce pure ω3-FAs. This process uses differences in melting points of various pure FAs or solutions [8]**.** Extraction with supercritical fluids is a relatively new method used in food and pharmaceutical processes. Use of supercritical fluids for the extraction and purification of ω-FAs from fish oils has already been reported [2,7]. Fish oil in the form of free FAs and esters is extracted using supercritical CO_2 to produce EPA and DHA concentrations [9,10]. Another way to concentrate and purify ω-FAs is chromatography. The HPLC, silver resin chromatography and supercritical liquid chromatography have been used to concentrate ω-FAs from natural sources [11]. Fractional distillation is another simple process for separating ω3-FA mixtures under low pressures. This method uses differences between boiling points and molecular weights of FAs under high temperature conditions (180-250 °C) and low pressures (0.1-1.0 mm Hg) [12]. However, using relatively high process temperatures and low pressures may include high operating costs, as well as thermal degradations of ω3-FAs. Adding urea is one of the most effective and simple techniques for the concentration and purification of ω3-FAs from natural sources. The complex formation of direct-chain urea and SFAs is a valuable and widespread separation method for FA or ester compounds [13]. When the highlighted separation methods are used alone, they only can concentrate and purify ω-FAs to a limited extent. Therefore, two or more methods are needed to produce high-concentrations ω3-FAs. A simple and inexpensive method involves soaping the wet biomass and cooling and adding urea in succession after trans methylation. In selection of an appropriate method for ω3-FAs purification from microalgal FAs, costs of the process and industrialization must be considered as well as the extraction efficiency. Hence, the urea complex method can extract fully economical FAs with satisfying efficiencies [14]. Therefore, methods of ω3-FAs extraction from *Chlorella vulgaris* microalgae were investigated and optimized in this study.

2. Materials and Methods

2.1. Microalgal cultivation

Inoculation was carried out using a native species of *Chlorella vulgaris* microalgae provided by the National Center for Aquatic Processing Research, Bandar Anzali, Iran, at a rate of 10% in Zarrouk culture media consisting of (Part A g l^{-1}) 16.80 g of $NaHCO_3$ and 0.50 g of K_2HPO_4; (Part B g l^{-1}) 0.50 g of $NaNO_3$, 1.00 g of K_2SO_4, 1.00 g of NaCl, 0.20 g of $MgSO_4{\cdot}7H_2O$, 0.08 g of EDTA-$Na_2{\cdot}2H_2O$, 0.04 g of $CaCl_2{\cdot}2H_2O$ and 0.01 g of $FeSO_4{\cdot}2H_2O$; (Part C: trace element Mixture A mg l^{-1}) 2.86 mg of H_3BO_3, 1.810 mg of $MnCl_2{\cdot}4H_2O$, 0.222 mg of $ZnSO_4{\cdot}7H_2O$, 0.015 mg of $MoO_3{.}H_2O$ and 0.074 mg of $CuSO_4{\cdot}5H_2O$ (total volume of 1.0 ml l^{-1}); (Part D: trace element Mixture B mg l^{-1}) 22.9 mg of NH_4VO_3, 47.8 mg of $NiSO_4{\cdot}7H_2O$, 17.9 mg of $NaWO_2$, 4.2 mg of $Ti_2(SO_4)_3{\cdot}6H_2O$ and 4.4 mg of $Co(NO_3)_2{\cdot}6H_2O$ (total volume of 1.0 ml l^{-1}) [15]. This is one of the most commonly used media for *Chlorella vulgaris*. There were two reasons for choosing the culture media, including its low nitrogen content and type of nitrogen source, which increased biomass and lipid content. Nitrogen source of the Zarrouk media included sodium nitrate, which produced more biomass and lipid content than the nitrogen sources such as urea and ammonia. The reason why sodium nitrate promoted a better microalgal growth is that nitrogen and sodium were important nutrients for microalgae and sodium nitrate could provide the two nutrients to microalgae [16]. The culture was incubated at 30 °C with agitation at 130 rpm and a light intensity of 2500 lux. Cultures were optimally grown. A culture medium with no microalgal inoculations was used as control. Photo-bioreactors were manufactured using 5-liter plastic gallons. Air compressor equipped with CO_2 cylinders was used for aeration. Spargers was used to better distribute air inside the photobioreactor, which was connected to the air compressor via a silicone hose (Fig. 1). An aeration rate of 0.5 vvm with 3% CO_2 was continuously provided for all treatments. Nearly 3 l of the culture media with pH 7-7.5 were poured into each photobioreactor and 300 ml of the microalgal solution (10% inoculation) were added to the bioreactors. Furthermore, a control sample of no microalgal inoculation was used. Then, photobioreactors were exposed to sunlight and the growth rate of microalgae was assessed regularly.

Figure 1. Images of photobioreactors

2.2. Assessment of microalgal growth rates and plotting growth curves

Turbidity assessment method was used to measure microalgal growth. In this method, 10 ml of the culture media were removed daily and their turbidity was assessed using spectrophotometer at 600 nm. Based on the absorbance values, growth curves were plotted.

2.3. Assessment of moisture contents in concentrated microalgae

Dry weight method was used to assess moisture contents in concentrated microalgae. Briefly, 1 g of the concentrated microalgae was weighed (W_1) and poured into a pre-weighed microtube (W_2). The microtube was incubated at 60 °C for 24 h. After the sample was completely dried, the microtube was weighed (W_3). From the difference in weight of the empty microtube and the microtube with dried micro-algae (W_3 - W_2), weight of the dried microalgae (W_4) was calculated. The following equation was used to calcu-late proportion of water in the concentrated micro-algae.

$$\text{Moisture (\%)} = \left[\frac{(W1 - W4)}{W1}\right] \times 100$$

2.4. Assessment of dry cell weights

Briefly, 1 g of the sample was poured into a pre-weighed microtube (W_1) and incubated at 105 °C. The sample weight was continuously assessed to lose all moisture and reach a constant weight (W_2). The dry cell weight was calculated using the following formula:

$$\text{Dry cell weight} = W1 - W2$$

2.5. Extraction of ω-fatty acids

There are several methods to break down the microalgae cell walls, increasing the efficiency of lipid extraction. In this study, ultrasound technique was used as a high and conventional method in most laboratory studies for cell wall disruption [17-20]. In this study, Nissei Ultrasonic Bath (NS-300 Model, Nissei, Japan) was used to collect the lipid content and ω3-FAs at 28 kHz frequency and 300 W power for 30 min. For 1 g of wet microalgae with a moisture content of nearly 65%, 5 ml of distilled water (DW) were used as solvent [15]. Samples containing DW and wet-microalgae were poured into 50-ml centrifuge tubes and inside the device and treated with ultrasound for 30 min. After cell wall disruption, samples were centrifuged at 3000 ×g for 5 min at 25 ºC and the supernatant was removed. Then, solvent extraction of lipids was carried using standard Bligh and Dyer protocol [21].

2.6. Assessment of extracted lipid contents

A volume of 1116.5 ml of ethanol 96% (by volume) and 23.5 g of KOH were added to 93.8 g of wet biomass (collected from 30 l culture), corresponding to 23.3 g of dry cell mass and 20.6 g of ash-free dry mass. The mixture was incubated using orbital shaker at 100 rpm and 20 °C overnight. Then, 100 ml of DW were added to the mixture followed by several hexane extractions (5 × 200 ml) to separate unsaponifiables. The hexane used in all steps (saponification, transmethylation, winterization and urea complexation) contained 0.01% (by mass per volume) of butylated hydroxytoluene to prevent lipid degradation. The hydroalcoholic phase containing the soaps was acidified to pH 1 by adding hydrochloride solution (1:1 by volume, 37% HCl). Then, free FAs (FFAs) were recovered by several extractions (8 × 200 ml) using hexane. The organic phase containing FFAs was dried using anhydrous sodium sulphate and the solvent was evaporated at 35 °C using vacuum rotary evaporator (Fig. 2).

2.7. Selection methods of ω3-fatty acid purification

For the purification of UFAs such as ω3-FAs, the urea complex was combined with winterization. Since FAs should be converted to methyl ester or ethyl ester before the urea complex, the methyl ester method is described.

2.8. Methyl esterification

As the urea complex with methanol was subsequently used, the achieved FAs extracted by the process were converted to methyl ester and entered the urea complex. The FFAs were methylated according to Mendes et al. [22] by adding 465.6 ml of a methylation mixture of methanol and H_2SO_4 (49:1 by volume) to the extracted FAs and heating at 80 °C for 1 h. After cooling the mixture to room temperature, 232.8 ml of water and a similar volume of hexane were added to the mixture and the upper hexane layer was removed and dried using anhydrous sodium sulphate. The solvent was evaporated at 35 °C using vacuum rotary evaporator and *n*-hexane was added to the remnant (V = 2.46 ml) (Fig. 2) [22].

2.9. Combined extraction method (winterization-urea complex)

In the combined extraction method, the methyl ester FAs achieved from the previous step were stored at -18 °C overnight. After crystallization, the water phase was isolated. Based on the quantity of FAs from the gas chromatography analysis, the urea complex process was carried out with ratios of urea to FA of 3.5 and methanol to urea of 3.4 and a crystallization temperature of 20 °C. The mixture was heated at 60-65 °C and stirred until the solution became clear. The urea complex was achieved by cooling the solution at 20 °C overnight, followed by an ultra-centrifugation at 10,000 ×g and 20 °C to separate urea from non-urea complexing fractions (Fig. 2).

2.10. Gas-chromatography analysis of ω3-fatty acids

Methyl ester FAs were analyzed using gas-liquid chromatography (GLC). Separation of FAs was carried out on a 0.32 mm × 30 m fused silica capillary column with helium as carrier gas at a rate of 3.5 ml min^{-1}. The initial column temperature was programmed at 200 °C for 8 min, increasing by 4 ºC/min to 240 ºC and holding at the maximum temperature for 8 min. The injector and detector temperatures included 250 and 280 ºC, respectively. The split ratio was 1:50. Detection was carried out using flame ionization detector at 260 °C with hydrogen rate of 40 ml min^{-1} and air rate of 400 ml min^{-1} by 25 ml min^{-1} make-up gas. Peak identification was carried out using known standards. Methylheptadecanoate ester was added as internal standard.

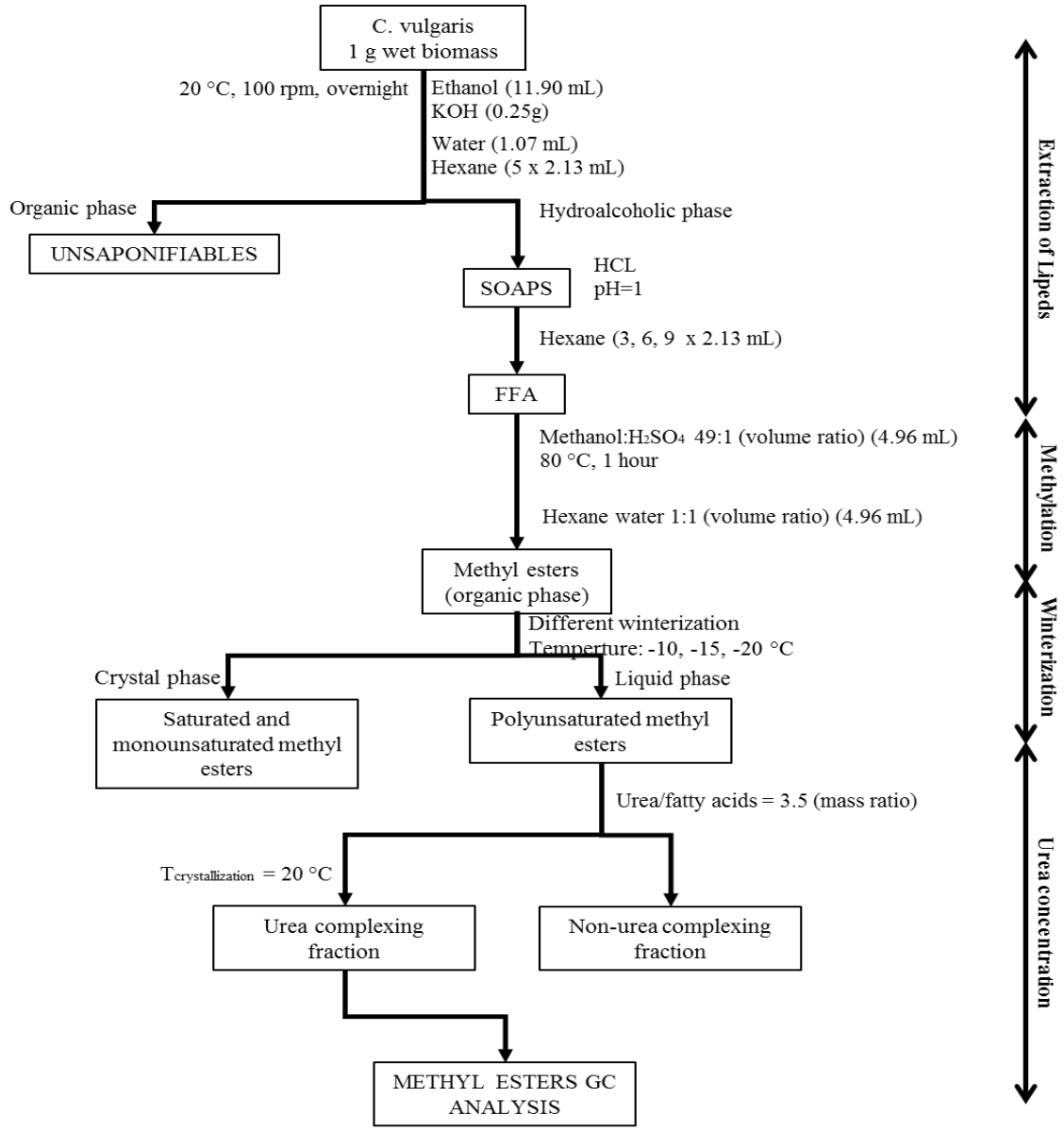

Figure 2. Flowchart of ω3-fatty acid recovery using *Chlorella vulgaris*

2.11. Experimental design

Experiments were designed using Design Expert 10 software and response surface methodology. Central composite design was used for setting up and analysis. Three variables of frequency of hexane extraction and winterization temperature and time were selected. In this study, 4 to 8 times of extraction with hexane, winterization temperature of -10 to -20 ºC, and winterization time of 10 to 20 h were selected as lower and higher levels of the variables, respectively. Values of the variable are presented in Table 1 and a total of 15 runs presented by the software are described in Table 2.

Table 1. Parameters and levels of the optimization

Parameter	upper (+1)	lower (-1)	+α	+α	Central point
Winterization temperature (ºC)	-20	-10	-22	-8	-15
Winterization time (h)	20	10	22	8	15
Frequency of hexane extraction	8	4	10	3	6

Table 2. Designed experiments for the optimization of ω3-fatty acid extraction using combined method

Run	Frequency of hexane extraction	Winterization time (h)	Winterization temperature (°C)
1	9	20	-20
2	6	22	-15
3	10	15	-15
4	6	15	-15
5	6	15	-22
6	6	15	-15
7	6	8	-15
8	9	10	-10
9	6	15	-15
10	6	15	-15
11	4	10	-20
12	6	15	-15
13	4	20	-10
14	3	15	-15
15	6	15	-8

3. Results and Discussion

3.1. Microalgal cultivation

The first reason for choosing *Chlorella vulgaris* in this study was that the microalga included the highest production of biomass within the microalgae. The second reason was that although the lipid content of this microalga was lower than that of some other species such as *Nannochloropsis*, more biomasses could be achieved from *Chlorella* in a certain volume (e.g., 1 l) of the culture media. Therefore, the algal lipid production efficiency was higher or equal to high-lipid content species. Although *Nannochloropsis* species were mostly isolated from marine environments, goal of the current study was to produce ω-FAs from freshwater algal species [23,24]. Microalgae culture was initially cultured in 2-l flasks and incubated using shaker-incubator. Light exposure was carried out using several LEDs with a light intensity of 3,000 lux. Light intensity was adjusted by increasing the number of LEDs. Light intensity affected balances between the structure of membranes and the storage fats, causing oscillations in distribution of lipid classes and changes in distribution of FAs [15,25]. After inoculation of the preculture media in photobioreactors and exposure of the cultures to sunlight, turbidity was assessed by sampling at specified time intervals (Fig. 2). After the growth period was over and the microalgae entered the stationary phase, the microalgae must be separated from the culture media. Microalgae proliferation might be stopped if live microalgae were not isolated from the media and live cultures began to die. Typically, microalgae grew well after 5-7 d in photobioreactors; hence, they could be collected at the first point of emergence into the stationary phase. In experiments, the microalgae eventually grew to an absorbance of 1.05 (Fig. 3). Based on the curve, the first two days belonged to the lag phase. After the Day 2 until the Day 5, specimens were in logarithmic or growth phase. After the Day 5 until the Day 7, the rest phase could be seen. On Day 5 or 6, aeration system could be turn off and the microalgae could be collected. In this study, the algal cells were collected at the beginning of Day 6. Then, the samples gradually entered the death phase.

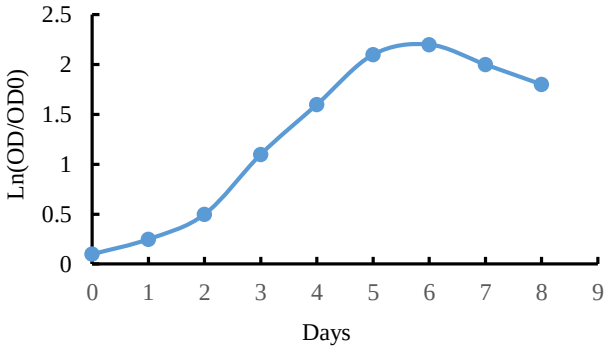

Figure 3. Growth carve of *Chlorella vulgaris*

3.2. Optimization of ω3-fatty acid extraction using combined technique

In ω3-FAs extraction process, optimization testing provided by the software with respect to the minimum and the maximum values for the effective factors are shown in Table 1 and the achieved values in Table 3. In this study, number of extractions with hexane and winterization temperature and time were considered as three effective factors. To optimize these factors, the RSM-CCD section of Design Expert 10 software was used. As shown by data from Table 3, the highest quantity of ω3-FA extraction belonged to Experiment 5 with time of 15 h, temperature of -22 °C and six times of hexane extraction. Table 4 shows variance analysis of the data. As presented in the report, this model, including F-value = 145.77, R^2 = 0.9962 and *p*-value < 0.0001, was quite stable and significant.

Table 3. Results of ω3-fatty acid extraction optimization using combined process

Run	Frequency of hexane extraction	Time (h)	Temperature (°C)	Amount of ω3-fatty acids extraction (mg g^{-1} of fatty acids)
1	9	20	-20	31
2	6	22	-15	27
3	10	15	-15	30.6
4	6	15	-15	36.5
5	6	15	-22	43
6	6	15	-15	36.4
7	6	8	-15	29.25
8	9	10	-10	23.85
9	6	15	-15	25.7
10	6	15	-15	34.8
11	4	10	-20	34.2
12	6	15	-15	36
13	4	20	-10	20
14	3	15	-15	27.225
15	6	15	-8	22

The model considered for these responses was a quadratic model. The overall ability of the model was typically described by measuring the coefficient of R^2, including a measure of the degree of adaptability of the model. However, the R^2 coefficient alone was not enough to validate the model. Therefore, the variance analysis was carried out for the model (Table 4). In this model, the parameters of A, B, C, AC, A^2, B^2 and C^2 were important due to their *p*-values of less than 0.05. Relative importance of the parameters and interaction between the parameters in the final model were presented by the coefficient of influence of each of them in the model. Based on the analysis by the software and data from the experiments, the proposed model of software was a quadratic model. The proposed equation for this model was as follows:

ω3-FA recovery = -89.03216 + 11.18596 * number of hexane extraction - 4.33212 * temperature (°C) + 6.07212 * time + 0.043869 * number of hexane extraction * temperature (°C) - 0.16423 * number of hexane extraction * time + 0.042344 * temperature (°C) * time - 0.58278 * number of hexane extraction ^ 2 - 0.064458 * temperature (°C) ^ 2 - 0.15374 * time ^ 2

As results of F-values in analysis of variance showed, winterization temperature included the greatest effects on extraction efficiency followed by the time and number of extractions with hexane, respectively. In a study by Mendes et al, temperature included great effects on the extraction [22]. The reason included that the basis of winterization process was the insolubility of SFAs at low temperatures to increase the quantity of UFAs.

3.3. Effects of extraction frequency with hexane

Based on Fig. 4, number of extractions with hexane included minor effects on the extraction efficiency. By increasing the number of extractions from 4 to 6 times, the extraction rate increased with a steep slope. By increasing the number of extractions, extraction efficiency did not change and mildly decreased, indicating that the maximum quantity of ω3-FAs in six-time extractions with hexane was introduced into the organic phase and the remaining ω3-FA content was low. By increasing the extraction frequency, no significant changes were seen between the concentrations of ω3-FA extracts. Approximately, an 18% change was seen in extraction efficiency between the optimum and the minimum points.

Table 4. Summary of the analysis of variance

Std. Dev.	0.65	R-Squared	0.9962
Mean	31.17	Adj R-Squared	0.9894
C.V. %	2.09	Pred R-Squared	0.9432
PRESS	31.90	Adeq Precision	43.73

3.4. Effects of temperature

As shown in Fig. 5, efficiency of ω3-FAs extraction decreased with increasing temperature at a steep gradient; hence, there were more than 40% decreases in efficiencies between the optimum and the minimum points. Based on the coefficient of influence of the factors in the equation and F-value of the factors, temperature included the greatest effect on the extraction efficiency. Based on the results of Experiment 5, the extraction did not change from -20 to -22 °C and -20 °C could be considered as the optimal temperature. The reason for decreases in extraction efficiency with increasing temperature is thus interpretable, as deceases in temperature caused decreases in crystallization of SFAs and hence concentrations of UFAs and ω3-FAs decreased. At a temperature range of -15 to -20 °C, the highest winterization of SFAs occurred. Furthermore, lack of thermal effects of -22 to -20 °C was due to the 95% concentration of UFAs at -18 to -20 °C. In another study, -10 °C was reported as the optimal temperature [26]. Moreover, -18 °C was set up in a study by Mendes et al. The necessary temperature highly depended on the composition of microalgal FAs [22]. Higher quantities of SFAs caused further cooling and winterization times.

3.5. Effects of winterization time

As seen in Fig. 6, winterization time included the minimum effect on the extraction efficiency of ω3-FAs and the difference between the optimum and the lowest point was less than 10%. Increasing the time from 10 to 15 h with a sloping gradient led to increases in extraction, which could be neglected when comparing to increases in extraction costs due to increases in extraction times. The reason could be that when the temperature reached -20 °C, most of the SFAs were frozen and UFAs were concentrated and the extra time did not further help this process. Time between 10 and 12 h could be optimal. Decreasing the extraction efficiency by increasing the time from 15 to 20 h might be due to the effects of long-term suppression at low temperatures on UFAs that could degrade the chemicals and decrease concentrations of UFAs. Another study reported an optimum winterization time of 14 h, which did not save much times [22]. In contrast, a study by Wanasundara and Shahidi in 1999 showed that a 24-h time was optimal. This difference was linked to the type of algal strain [27].

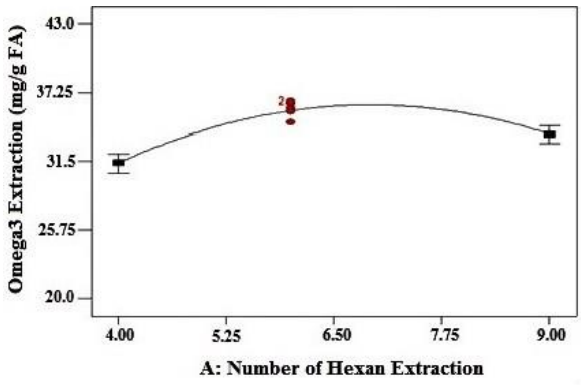

Figure 4. Frequency effects of hexane extraction on yield of ω3-fatty acid extraction in the combined process

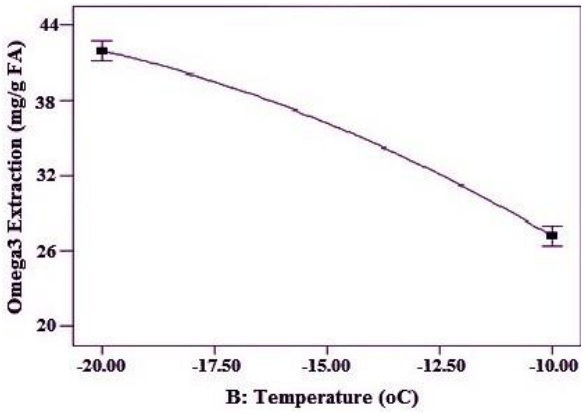

Figure 5. Temperature effects on ω3-fatty acid extraction efficiency in the composite process

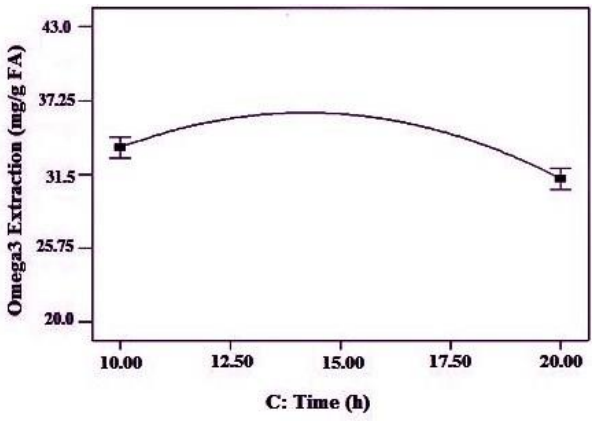

Figure 6. Effects of winterization time on ω3-fatty acid extraction efficiency in the combined method

3.6. Interaction effects of extraction frequency with hexane and winterization time

Based on the results from the analysis of variance, only the parameters of the frequency of extraction with hexane and winterization time included significant interactions, which were represented as surface shape charts. In fact, surface-level graphs were three-dimensional charts plotted as functions of two independent variables in experiments, while other variables were plotted on a constant level. Based on the curve of Fig. 7, with increasing the extraction time from 10 to 15 h and the frequency of extraction with hexane from 4 to 8 times at a constant temperature of -15 °C, efficiency of the extraction increased with a steep gradient. Although the two factors alone included little effects on the extraction efficiency, their interaction included significant effects on the extraction rate. As shown in the curve of Fig. 7, the two parameters at intervals of 17.5 > time > 15 and 8 > the number of extractions with hexane > 6 were in their optimal range. This interaction increased the extraction time more than that the single factor mode did. In this interaction, similar to the single factor mode, effects of the number of extractions with hexane were greater than those of the time. In particular, the maximum extraction rate (43 mg) was achieved at -20 °C, 12 h and six times of extraction with hexane. As soaping and methylation processes were generally carried out on the cells during the winterization process, these processes were more useful and cost effective than those carried out on FAs or lyophilized cells [27]. To check the profile of ω3-FAs produced by gas chromatography analysis, the analysis was carried out under optimal conditions (Fig. 8). Based on the area under the curve, EPA and DHA included 87.22 and 12.77%, respectively.

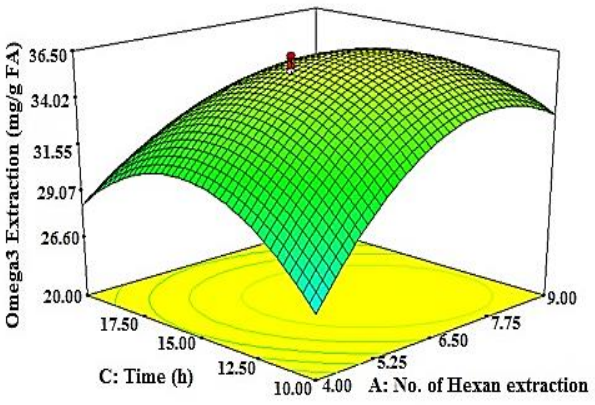

Figure 7. Effects of number of hexane extraction and winterization time on the extraction efficiency of ω3-fatty acids

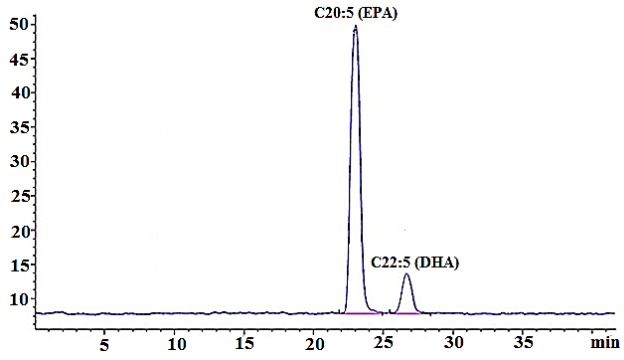

Figure 8. Gas chromatography analysis of the recovered ω3-fatty acids

4. Conclusion

In this study, extraction method was optimized using urea complex and winterization and three factors were investigated. Based on the combined method, three factors of temperature and times of winterization and number of extractions were optimized. Winterization temperature included the highest and the number of extractions included the minimum effects on the extraction of ω3-FAs using combined method. The optimal time of hibernation was 10 h, which was an appropriate time for this process. This can greatly decrease the cost and time needed to complete the process. Despite the fact that studies on combined process with various microalgae have shown that this method is more appropriate than the urea complex, results of this study did not show significant differences in extraction rates. Since processes of soaping and methylation are carried out on cells in the method of winterization, it can be more efficient and beneficial than working on FAs or lyophilized cells, such as that of the urea complex method. Since the urea complex method is carried out within 16 h, it is much more appropriate than the combination method in terms of extraction speed, which needs at least 26 h of extraction time.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflicts of interest.

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بهبود فرایند بازیافت اسیدهای چرب امگا-۳ از گونه‌های بومی *کلرولا ولگاریس* با استفاده از روش یکپارچه

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چکیده

سابقه و هدف: کبد و بافت ماهی و برخی ریزجلبک‌ها غنی‌ترین منابع اسیدهای چرب امگا-۳ در طبیعت می‌باشند. سالانه، بیشتر از ۴/۲ بیلیون تن جلبک با اهداف گوناگون کشت می شود. بنابراین، توسعه راه‌های نوآورانه به‌منظور استخراج مقادیر بیشتر اسیدهای چرب امگا-۳ از بافت ریزجلبک هدفی ارزشمند به‌شمار می‌رود. در این مطالعه، روش‌های گوناگون خالص‌سازی و استخراج اسیدهای چرب امگا-۳از کونه‌های بومی ریزجلبک‌ها باهم مقایسه شده‌اند.

مواد و روش ها: برای استخراج و خالص سازی اسیدهای چرب امگا-۳ از روش ترکیبی (کمپلکس اوره و زمستانه-کردن) استفاده شد. روش ترکیبی، با مطالعه سه عامل درجه حرارت زمستانه‌کردن، مدت زمان زمستانه‌کردن و تعداد دفعات استخراج با هگزان در ۴ سطح گوناگون و انجام ۱۵ آزمون طراحی شده با نرم افزار Design Expert 10.0 بهینه‌سازی شد.

یافته‌ها و نتیجه‌گیری: نتایج بهینه‌سازی شامل ۱۰ ساعت برای مدت زمان زمستانه‌کردن، ۲۰- درجه سلسیوس برای درجه حرارت زمستانه‌کردن و ۶ بار استخراج با هگزان بود. در شرایط بهینه، بیشترین میزان اسیدهای چرب امگا-۳ حاصل از *کلرولا ولگاریس* در رطوبت ۶۵ درصد، ۴۳/۶ میلی‌گرم بر گرم اسیدچرب بود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

تاریخچه مقاله

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واژگان کلیدی

- امگا-۳
- ریزجلبک‌ها
- استخراج با حلال
- کمپلکس اوره
- اسیدهای چرب غیراشباع
- زمستانه‌کردن

***نویسنده مسئول**

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